



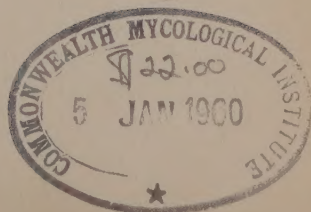




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# Plant Pathology

*An Advanced Treatise*

*Edited by*

J. G. HORSFALL AND A. E. DIMOND

*The Connecticut Agricultural Experiment Station  
New Haven, Connecticut*

VOLUME I

**The Diseased Plant**



1959

ACADEMIC PRESS, New York and London



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ACADEMIC PRESS INC.

111 FIFTH AVENUE

NEW YORK 3, N. Y.

*United Kingdom Edition*

Published by

ACADEMIC PRESS INC. (LONDON) LTD.

40 PALL MALL, LONDON S.W. 1

*Library of Congress Catalog Card Number 59-7684*

PRINTED IN THE UNITED STATES OF AMERICA

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## PREFACE

The purpose of this treatise is to present an integrated synthesis of the parts of plant pathology. It is designed for the creative worker, not for the beginning student. Many books discuss the specific diseases of plants and the control measures for them. This work does not. Rather it treats of the concept of disease, not of diseases. It is concerned with the normal pathways that are obstructed in sick plants, how the pathogen brings about dysfunction, and how the host plant reacts to the pathogen. This work deals also with the logistics and the strategy of disease and its combat.

A single chapter may deal with lettuce and elm trees, with viruses and nematodes, with insecticides and fungicides. The underlying principles of plant pathology involve them all.

The present treatise could not have been written 25 years ago. The many scientific discoveries in plant pathology, in biology, in physics and chemistry generally, have contributed to the present status of our knowledge. The editors and the authors, working together, have attempted to draw these together, in the words of Brierley (*Ann. appl. Biol.* 33: 336-337, 1946), to form "a consistent body of theory which correlates the facts into a logical and explanatory system."

The editors hope that the treatise will appeal to all who are interested in a theoretical treatment of plant pathology, to those interested in the broad ecological relationships among organisms, and to research workers and advanced students of applied biology.

Volume I deals with the diseased plant, with the scope, importance, and history of plant pathology; with pathological processes and defense devices; with predisposition and with therapy of the diseased plant.

Volume II focuses the attention of the reader on the pathogen itself and on techniques inhibiting it. It deals also with parasitism and reproduction and with pathogenicity and its inhibition.

Volume III deals with the diseased population of plants, with epidemics and their control. This, the public health aspect of plant pathology, has hitherto received short shrift. Volume III gives major consideration to production and to dispersal of inoculum, to epidemics and their forecasting, to control of inoculum at the source, in transit, and in the infection court. A final section deals with the genetics of resistance to disease and problems of the plant breeder.

This is an international treatise. Its preparation would not have been possible without the willing and able participation of many people from all over the globe. The framework on which the chapters hang was con-

structed with the aid and advice of a distinguished group of plant pathologists who comprise our advisory board. Thus, we are grateful to F. C. Bawden of Britain, R. Ciferri of Italy, Ernst Gäumann of Switzerland, George McNew of the United States, T. S. Sadasivan of India, G. G. Taylor of New Zealand, I. Uritani of Japan, and C. E. Yarwood of the United States. The chapters have been written by an equally distinguished group of plant pathologists, who also bring international representation to the work.

The authors devoted their time assiduously to the preparation of manuscripts, despite the pressure of their regular jobs. Manuscripts generally were completed before the necessary deadlines. The subject index was prepared by Mrs. Raimon L. Beard. Without the help of our able secretary, Miss Lois Pierson, this project would have been difficult. She handled the heavy correspondence, retyped many corrected manuscripts, checked proofs and was helpful in many other ways.

To further embellish our gratitude to all would be to degrade one of the most elegant and simple sentences in the English language, "We thank you!"

J. G. HORSFALL  
A. E. DIMOND

*May, 1959*  
*New Haven, Connecticut*

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## CHAPTER 1

# Prologue

## The Diseased Plant

J. G. HORSFALL AND A. E. DIMOND

*The Connecticut Agricultural Experiment Station, New Haven, Connecticut*

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## I. INTRODUCTION

Given three volumes in which to set out the principles of plant pathology, we found that the subject dissected easily into the required three segments: The Diseased Plant; The Pathogen; The Diseased Population.

This distinction has not been emphasized hitherto. In fact, Stakman and Harrar (1957) in their excellent text say that "Diseases of individual plants are relatively unimportant, and plant pathology is therefore essentially a community aspect, or plant public health science."

The point made by Stakman and Harrar is true for the art of plant pathology but it is not as true for the science of plant pathology. There we need to understand the nature of disease in the individual as much

as we need to understand the nature of disease in a population of individuals.

It seems strange that despite Stakman and Harrar's dictum, little effort has been made to treat disease in the individual separately from disease in the population.

## II. BACKGROUND FOR VOLUME I

To open the treatise with the diseased plant rather than with the pathogen is somewhat "out of step" with the times. The temptation is very strong to follow the etiological approach. We were raised in that school and this is surely the classical approach. Most textbooks have been organized around the microbial agents of disease. They carry such titles as Bacterial Diseases of Plants, Virus Diseases of Plants. Even if the title is as simple as Plant Pathology, the inner organization is tied to the microbes and the chapters read, Diseases Caused by Ascomycetes, Diseases Caused by Basidiomycetes, etc.

We start with the diseased plant rather than with the pathogen because we are offering a treatise on plant pathology—not a treatise on plant pathogens. The diseased plant is the central theme. In fact, the diseased plant was the one theme prior to promulgation of the microbial theory of disease in plants by De Bary (1866) and Berkeley (1857) about 100 years ago. Zallinger (1773) entitled his book "*De Morbus Plantarum*" and Unger (1833) entitled his "*Exantheme der Pflanzen*."

We begin as the science began, with The Diseased Plant.

Almost 20 years ago Morstatt (1929), a German, developed some resounding arguments in favor of developing the concept of the diseased plant rather than that of the so-called causal agents. Morstatt contended that the rise of the pathogen school has seriously distracted attention from the diseased plant and its malfunctions. It has pushed the diseased plant from the center of the stage and substituted the pathogen which is only of ancillary importance. Thus, it has arrested the development of plant pathology as a science.

Morstatt further contends that the dominance of the pathogenicists has fractured phytopathology into applied mycology and applied entomology, and the applied aspects of virology and nematology. This has further depressed development of the whole science.

We agree with Morstatt. One hundred years since De Bary and Berkeley seems long enough.

Whetzel (1928) says: "There must be no evasion of the fact that the diseased plant is the central figure in the phytopathological drama. . . . The disease concept must condition and rationalize all other concepts

of our science. . . . The causal organism, where such is involved, must be relegated to its distinctly secondary role."

Pathogens, of course, are important, we all agree, and they will be assigned a whole volume. But, even so, they will be reduced to the ancillary position that they truly occupy in the whole science.

### III. WHAT IS PLANT PATHOLOGY?

Every field of science has its own list of stock words, its own jargon, and plant pathology is no exception. Humpty Dumpty's dictum in Alice in Wonderland is a classic for all users of the jargon. "When *I* use a word," Humpty Dumpty said in rather a scornful tone, "it means just what *I* wish it to mean—neither more nor less."

What then does the term plant pathology mean? Presumably, what plant pathologists want it to mean. The term pathology like numerous others in science stems from the Greek. It is no idle jest that "the Greeks had a word for it." Generally they did. The Greeks were astute observers of nature and they coined words to name most of the broad concepts about things.

The word derives from two Greek words: *pathos*—suffering—and *logos*—to speak. Plant pathology, then, is a discourse on the suffering plant. It covers the field of the suffering plant. A distinguished botanist has expressed surprise to us that plants really suffer. Presumably, to him suffering must imply pain and anguish, and plants do not suffer pain or anguish. Still, we think that the word suffering is not stretched too much when we use it for plants. *Pathos* occurs in other parts of our language. Sympathy, for example, means suffering together.

The field of endeavor that we call plant pathology encompasses both the art of treating the sick plant and the science of understanding the nature of the diseased plant. This has created some confusion as Horsfall (1959) has suggested.

Reduced to their bare bones, art is doing; science is understanding.

Very often plant pathology is called an applied science. This is a confusing misnomer. The very verb "to apply" means to put to a particular use. Thus, applying knowledge is an art. The knowledge may have come from the efforts of scientists, but its application is an art. An applied science then is a misnamed art.

One finds this same misuse of the word science, for example, in the remark, "He has reduced bridge playing to a science." This sentence means that he has reduced bridge playing to an art. The mathematical study of bridge playing aimed to deduce the odds on a finesse, for example, is a science. The use of the finesse to trap a king, is an art.



Using a finesse at just the proper time is a fine art. To use science to mean art is to create confusion.

The history of the science and the art of plant pathology is interesting and significant for an understanding of the interrelations between the two.

If one had to pick a date for the beginning of the science of plant pathology, he might choose the middle of the last century when De Bary (1866) and Berkeley (1857) made the breakthrough that clarified the role of the pathogen in disease. Prior to that, the cart was put before the horse. The pathogen was considered an excrescence from diseased tissue.

Once the proper sequence of causality was established, the science of plant pathology exploded in its development.

When this happened, governments discovered that the new knowledge of disease gained by the "impractical theorists" could be put to work. To a politician knowledge is power, and the pathologists' knowledge provided power. Governments all over the world set up so-called experiment stations and ensconced plant pathologists in them.

As soon as this was accomplished, the art of plant pathology was born: the art of diagnosis and treatment of the diseased plant. Thus, the art followed after and sprang from the science.

The art, like the science before it, has also shown an explosive rise in importance.

Human medicine is the analogue of plant pathology. Here, in contrast to plant pathology, the art came first. Man has had medicine men to treat his ailments since primitive times. Because the medicine man deals with the art, he is called a practitioner and his business a practice. He is not called a pathologist. He does not study the nature of disease. He treats it.

The scientist is a latecomer in human medicine. He aims to understand the phenomena of human disease. He does not treat it. In fact, he is prohibited by law from treating it. He even has a different degree. He has a Ph.D. The practitioner has an M.D.

In medicine, M.D. men do do research; Ph.D's never do practice. This probably stems from the fact that the science grew out from the art and dragged some artists with it. In plant diseases, the scientist often must have a practice, circumstances often demand it. The full time practitioner, however, seldom does research except as a spare-time job. This probably stems from the fact that the art grew out from the science and dragged some scientists with it.

In medicine, there are not enough scientists to do all of the science. In plant pathology, there are not enough practitioners to do all of the

practice. Inevitably there is a flow from the high to the low potential, irrespective of direction.

Paradoxically, the science suffers in both cases, from an influx of inadequately trained people on one hand, and from loss of personnel on the other.

We may summarize by saying that the "discourse on the suffering plant" inevitably involves two phases: the science of learning and understanding disease, and the art of applying the knowledge to real life problems. This dual nature of the profession has created a certain amount of confusion especially because of the tendency to use the word science to cover nearly everything.

#### IV. WHAT IS DISEASE?

A suffering plant is diseased; what then is disease? This concept is even more difficult to reduce to a few words. On the other hand, a scientist or layman has a fair facility for understanding disease because he himself is diseased sometimes.

##### *A. Disease Is Not a Condition*

A disease is very often considered a condition, but disease is not a condition. A plant or an animal may be in a diseased condition. The condition, however, results from the disease and is not synonymous with it.

Condition is a symptom complex. The Germans call this a "Krankheitsbild"—a disease picture. Our medical colleagues call this a syndrome. A plant may be dwarfed and the leaves mottled and yellowed. We often refer to this as a mosaic disease. In doing so, we have named the disease by naming the chief and, perhaps, most obvious symptom. The disease is deeper than the symptom.

##### *B. Disease is Not the Pathogen*

Very often we confuse disease and pathogen. This derives from our microbiological heritage. Prior to Berkeley or de Bary, we would never have made this mistake. This is a late-coming bit of confusion.

Since the beginning of agriculture, our cereals and many other crops have been smitten with a black blasting of the grains. This symptom has a name in every language—smut, brand, charbon—all meaning black. In English we say wheat smut, barley smut, rye smut. There are loose smuts and covered smuts, flag smuts, and stripe smuts.

When mycologists arrived on the scene of plant pathology, they discovered that the smut diseases are caused by fungi that are related. They looked around for a name for this interesting group of fungi and

named the fungus from the name of the disease. They called them the Ustilaginales and this word comes from the Latin, *ustilare*, meaning to burn or to blacken.

This really confused and confounded the issue. We had the smut disease, then we had the smut fungus, and we slipped so easily, oh so easily, into the fallacy of equating disease with the pathogen. We say "loose smut of wheat is *Ustilago tritici*." We went through a similar course of events for downy mildews, powdery mildews, and many others.

Of course, the experts contend that they do not confuse the two.

Whetzel (1929) strongly protests against the influence wielded by mycologists. He says: "Many of the courses [and books. Eds.] today, masquerading under the name of plant pathology, are but mycology or a bastard progeny, resulting from an illegitimate cross between mycology and the art of plant disease control . . . no more striking evidence of its baneful influence can be asked than the prevalent practice of treating the pathogen and the disease as synonymous concepts." Whetzel continues: "I refrain from embarrassing you with quotations from your own publications. Few of us are innocent of this grossest of errors."

### C. Is Disease Catching?

Another widespread confusion derives from the phrase that a disease is "catching." Undoubtedly, this usage antedates the germ theory of disease. The black plague was catching in the Middle Ages, but St. Anthony's fire (ergotism) was not.

In a general sense, one throws a ball and another catches it. In the great American game of baseball, the one who catches the ball is a catcher. In this sense only the pathogen can be caught, not the disease. This is a usage that will not be stopped by any feeble words in this treatise. Disease will continue to be catching and the concept will continue to confuse the issue of what disease is.

Perhaps the most difficult pair of concepts of all to separate is injury and disease. Again every person probably senses the difference and, hence, it may not be necessary to go any farther. Still, an attempt to separate them may aid in throwing light on just what disease is.

### D. Disease Is Not the Same as Injury

Plant pathologists will sometimes spend an entire coffee break discussing whether or not a lawn mower or a cow can produce disease on the grass. Most will agree right away that a lawn mower produces injury and not disease. The plant surely suffers when the lawn mower removes a large part of the photosynthetic area. It grows less well. Still, we do not consider this a disease. The amateur woodworker knows that he is

injured when he cuts off his finger in his power saw, but he knows he is not diseased. If he gets a boil on his finger, he says it is diseased and not injured.

This suggests one significant point of difference between injury and disease. The power saw is in contact with the flesh for only a micro-second. The *Staphylococcus* pathogen remains in the boil until it is cured, or nearly so. In general, we consider we are diseased if the action of the causal agent is prolonged, and injured if it comes and goes suddenly.

#### *E. Disease Results from Continuous Irritation*

Thus, it seems fair to say that disease results from continuous irritation and injury from transient irritation.

#### *F. Disease Is a Malfunctioning Process*

Now, one last point. The standard question we ask our sick friend is, "What is the matter with you?" His reply is usually "My stomach is acting up," or "My head aches," or "My vision is blurred." These all contain a verb full of action. Something is functioning poorly, and hence, we come to the decision that disease is a malfunctioning process that is caused by continuous irritation. Of course, this process must result in some suffering. And hence, disease is a pathological process.

As near as we can determine, Ward (1901), later Morstatt (1929), and Whetzel (1929) ardently supported this view. This conception of disease was accepted by the Committee on Terminology of the American Phytopathological Society (See Reddick *et al.*, 1940) and by its counterpart committee of the British Mycological Society (Anonymous, 1950).

#### V. HOST OR SUSCEPT?

Whetzel (1929) coined the word "suscept" to denote the diseased plant. Suscept is an ugly word and it has not caught fire in the profession. His reasoning is probably sound and his idea would probably have been adopted had he coined a more euphonious term.

According to Link (1933), Ray (1688) was the first to apply the term parasite to mistletoe. Link says further that Duhamel in 1728 applied the term parasite to a fungus now called *Rhizoctonia crocorum* and proved experimentally that the fungus could live at the expense of the saffron bulb.

Whetzel argued that the discovery of living fungi in diseased tissue led promptly to the use of the couplet "parasite and host." This, according to Whetzel is a workable couplet as long as the connotation is used

to apply to the food relation which is the essential feature of parasitism, but it is not so workable a concept when disease results.

Whetzel agrees that pathogen is a good term for the disease inducer, but host is not satisfactory for the organism that suffers from the induced disease. He coined *suscept* meaning the organism that is susceptible to disease.

To us this still leaves something to be desired. *Suscept* does not exactly mean the diseased plant; it means only a plant likely to be diseased. One cannot be a host until his guests arrive. There is no word, presumably because there is no need for one, to describe one who invites guests. After his guests arrive, he is called a host.

*Suscept* does not really cover the case of the plant that is diseased after the arrival of the pathogen. Thus, we cannot accept the term *suscept*.

## VI. IMPACT OF PLANT DISEASE ON SOCIETY

In Volume I we treat disease and its effect on human history and human society. Since pathogens must eat too, they compete with us for our food supply and they sit down first at the table. The pathogens attack our food plants in the field long before the food comes to our tables. When conditions are right, they may consume so much food that the human population hungers. Very often the human population must change its food habits, however well fixed they may appear. Ten Houten cites several examples in Chapter 2 of this volume. Another striking example follows.

Eating habits of people are very difficult to change and yet plant diseases appears to be responsible for some deeply implanted eating habits. Carbohydrate is a very basic ingredient in the human diet. Variation in carbohydrate supply around the world is, therefore, of interest.

Why should Southerners in the United States eat corn bread (from maize), and Northerners wheat bread? The early settlers in both regions came from England where wheat was the "staff of life." Why did Southerners have to change to maize?

And why do northern Europeans eat wheat, middle Europeans eat rye, and Italians eat wheat? Why does the word bread mean wheat bread in England, rye bread in Germany, and wheat bread in Italy? Why does the word bread mean wheat bread in New England and corn bread in Virginia?

Horsfall (1956) has suggested that this situation stems from the ravages of wheat rust.

Wheat seems to be the preferred source of carbohydrate over much



of the world. We can assume that where rice, rye, and maize are substituted, wheat does not grow well enough to provide the needs.

Consider New England and Virginia. These were both colonized early in the 17th century by British people. Wheat was the staple source of carbohydrate in England in those days. Undoubtedly both sets of colonists brought wheat seed to the New World with them. Wheat became established in New England and nourished the colonists through the long and stubborn winter.

Wheat does relatively poorly in Virginia even now and we can assume that it did poorly in the 17th century. One primary reason for this is wheat rust. Wheat rust was rampant in Virginia, in the spring. Its destructiveness makes wheat growing a failing business in Virginia. Wheat rust does occur somewhat in New England today, and presumably did then too, but it is not catastrophically destructive.

The reason appears to be climate. Wheat rust revels in warm, wet weather in the spring. Virginia has warm, wet weather in the spring and its wheat goes rusty in a hurry. The weather in New England may be wet enough for rust, but it is too cold and so the wheat rusts relatively little.

In the United States, wheat grows without much rust in some climates as warm as that of Virginia. This is the climate of Texas and Oklahoma. The climate there may be warm enough, but it is too dry.

Therefore, going from New England to Texas, we have wet cool weather in New England in the spring; this means low rust incidence, high wheat yields, and wheat bread. As we go south, the temperature rises and rainfall remains high. This means high rust, low wheat yields, and corn bread. As we go west, the rainfall declines even in warm weather. This means low rust, high wheat yields, and wheat bread again.

If we go to Europe, we see that the pattern repeats itself. In Britain, the weather in the spring is cold, though rainy. This means low rust, high wheat yields, and wheat bread. In Central Europe, the spring weather is damp and warm. This means high rust, low wheat yields, and rye bread. If we go south to the Mediterranean littoral, the spring weather is warm and dry as in Texas. Rust is low, wheat yields are up, and spaghetti and meat-balls, or macaroni and cheese make our saliva flow.

All this suggests another interesting explanation.

During the Middle Ages, St. Anthony's fire ravaged the people. This was a strange disease. At first, the extremities, the fingers, and the toes tingled and then ached. The victim showed mental aberration and ran such a raging fever that the disease got the name St. Anthony's fire. Women aborted their babies. In severe cases, fingers and toes became

gangrenous and eventually sloughed off. Arms had to be amputated—and feet—all without anesthetic, too. It was a dreadful affliction.

Eventually, Thullier—a French physician—showed that St. Anthony's fire was caused from eating ergot, a fungus that affected the kernels of the rye that the people had to eat because they could not eat wheat which was too prone to black stem rust.

St. Anthony's fire began to decline in Central Europe during the 18th century and occurred only sporadically during the 19th and 20th centuries. This was due to the arrival of the potato in Europe through the perspicacity of Sir Walter Raleigh. Eventually, the potato replaced much of the rye because it produced cheaper carbohydrate than the cereals. Thus, eventually the ravages of St. Anthony's fire declined.

Of course, this led on into the late blight disease of potatoes and the Irish famine but this story is already so threadbare from hard usage that it is hardly worth space in a modern treatise on plant pathology.

## VII. CLASSIFICATION OF DISEASE

One sometimes hesitates to discuss classifications. The classification of phenomena runs a clear risk of stultifying the subject. Pigeonholing of facts often seems to reduce them to dry statistics. Moreover, facts often are very stubborn about classification. The pigeonholer often finds that his facts are too big or have the wrong shapes to fit into the neat cubicles he has designed for them. When they do not fit, the classifier may stew so much about nonconformity that he forgets his objectives.

### A. *The Theory of Classification*

Still, the human mind being human cannot digest the whole of a subject any more than the human stomach can digest the whole of a cow. Experience has amply shown that the subject and the cow must be reduced to chewable bites. These chewable bites are categories, and categories mean classification.

Therefore we must classify diseases. The bases for classification have swayed back and forth with the wind of opinion among plant pathologists. Prior to the arrival of mycologists, and their domination of the scene, diseases were classified by symptoms.

Zallinger (1773), for example, made five divisions much like those in animal diseases: (1) Phlegmasiae or inflammatory diseases, (2) paralysis or debility, (3) discharges or draining, (4) cachexia, or bad constitution, and (5) chief defects of different organs.

Plenck (1794) according to Hallier (1868) divided plant diseases into eight divisions: (1) *laesiones externae*, (2) *profluvia*, (3) *dibilitates*, (4) *cachexiae*, (5) *putrefractiones*, (6) *excrementiae*, (7) *monstrositates*,

(8) sterilities. Some of these classifications such as sterility and cachexia are based on disturbed physiological processes as indicated by Plenck's title for his book. This is a much more nearly modern arrangement of diseases than those that come between Plenck and the modern era. It resembles rather clearly that of Baldacci and Ciferri (1949) as discussed below. Plenck, however, assigned Latin binomials to the individual diseases. A tree trunk canker, he called *Carcinoma arborum*. Holmes (1939) has done the same with viruses, taking generic names from symptom types and specific names from hosts.

### B. The Microbial Classification

Then came the golden age of mycology and its pervading influence on plant pathology. For a hundred years, more or less beginning in the early part of the 19th century, when mycology dominated the scene, diseases came to be equated with microbial pathogens and diseases were thus classified on the basis of causal organisms.

Thaxter (1890), the famed mycologist, first plant pathologist at our laboratory, spoke disparagingly indeed of those who tried to classify diseases by such names as rusts, rots, blasts, and blights. Thaxter's plant pathology did not scintillate as brilliantly as his mycology. "Fungus disease," he said, ". . . is the term properly applied to a majority of the ailments among plants, which are commonly and loosely designated by such names as blast and blight, . . . scab, scald, and smut, all of which convey to the mind a more or less confused and inaccurate idea of what they are intended to distinguish." "Such diseases," says Thaxter, "are accurately known only by the scientific names [of] . . . the fungi which cause them. For example, onion smut in Connecticut is known as *Urocystis cepulae*." This is an amazing remark. Thaxter named the fungus and he was probably the only person in Connecticut who called onion smut *Urocystis cepulae*. Not even to this day do farmers call onion smut by any other name than onion smut. And Thaxter called plant pathologists "Squirt gun botanists"!!

Inevitably, the textbooks on plant pathology during the mycological era classified diseases just as Thaxter wished—diseases caused by bacteria, by Phycomycetes, Ascomycetes, Basidiomycetes. One need only cite Sorauer (1886); Duggar (1909); Brooks (1928); Ericksson (1930); Chester (1942); and Walker (1950 and 1957).

H. Marshall Ward (1901), the distinguished English plant pathologist, avoided this fallacy. He says (p. 121): "All disease is physiological in so far as it consists in disturbance of normal physiological function, for pathology is abnormal physiology. . . . That being understood I need not dwell on the common fallacy of confounding the fungus, . . . or

other agent with the disease itself, or of making the same blunder of confusing symptoms with maladies." Perhaps, Ward should have dwelt on it. It certainly needed it.

### *C. Necessity for Classifying by Processes Affected*

It is interesting, nevertheless, that Ward does not classify diseases by the processes they disturb. He classifies diseases in his book by the symptoms they cause—excrecences, necrotic diseases, monstrosities, etc. This reminds one of Plenck's classification 100 years earlier.

F. L. Stevens (1917) says: ". . . The diseases themselves, not the fungi need classification. . . ." Stevens attempted a classification of disease, but he did not define disease; and hence his classification is a little befuddled. His classifications are: "(1) wilt diseases due to mechanical stoppage of the vascular bundles by parasites . . . embolism, (2) disintegration of xylem structures . . . wood rots . . . , (3) diseases due to parasites wholly contained within the living protoplasm of the host cell . . . endocellular parasitism . . . (4) diseases due to parasites which draw their nutriment from living cells by haustoria . . . (7) diseases in which the host tissue is displaced . . . by fungus masses . . . mycosclerosis, (8) tumor, (9) necrosis."

One can see that Stevens could not really burst out of his mycological cage. He could see through the wire meshes, but he could not quite cut free. He still stews more about the parasite or the symptoms than about the pathological processes.

Baldacci and Ciferri (1949) have come close to our classification of diseases on the basis of the pathological processes involved. Their classification of diseases follows. Epiphytic, trophic (meaning starvation), hypnochereutic (meaning destruction of non-trophic tissues), auxonic (meaning growth), and degenerative (meaning tissue destruction). In our view, epiphytic diseases hardly fit here and Baldacci and Ciferri have neglected reproduction altogether.

We believe that disease is a pathological process, that it is abnormal physiology. To this concept we hold despite the fact that we were reared in the mycological era of plant pathology. We admit that our scientific genealogy stems from the Harvard school of Farlow who first taught plant pathology in America in 1875 and who introduced the mycological bias from his graduate days with de Bary.

Thus we must struggle as constantly as anyone against the Satanic temptation to confuse the fungus with the disease.

Similarly, we are in an agricultural experiment station confronted on all sides by the pragmatic pressures to confuse symptoms with disease.

Because of these conflicting pressures we also, like Ward and Stevens,



may zig when we should have zagged, but we have tried to arrange Volume I of this treatise to reflect the view that disease is a pathological process and we have cautioned the authors to avoid the diagnostician's-eye-view, to eschew the temptation to arrange things by symptoms. We believe that no body of theory of plant pathology can be built up on symptomatology per se.

We believe that one can build up a body of theory on pathological processes. In building up a theory of disease one uses symptoms as markers or as aids to assist in inferring the nature of the process that generates them.

An investigator in plant pathology, like scientists everywhere, must depend on his five senses in appraising plant disease. He can see symptoms. From these he must decide on the abnormal process that brings them on.

He sees that the plant wilts. His problem has only begun. Which physiological process is unbalanced—is it uptake of water, transport of water through the plant, or loss of water by the leaves.

#### *D. Diseases Must Be Named*

We name this one wilt disease because that is the major symptom. Having done so, we have to be careful not to confuse the thing with its name. This is a disease of the water supplying process. The plant thirsts.

The Connecticut farmer may call a disease onion smut, but he knows in his green thumb that onion smut starves the plant so much that he has nothing to sell. This is a disease of nutrition—the plant starves. The disease is malnutrition—the symptom and the name is smut.

#### *E. The Six Processes Classified Here*

Considering disease as a process, the chapters in Volume I on the diseased plant must be headed to indicate processes. Thus, a chapter heading has to be Tissue is Disintegrated, not necrosis. Necrosis is a symptom of tissue disintegration. We believe that in writing a chapter on disintegration an author is impelled to dig gold from such distant hills as wood and textile degradation. He might not be impelled to do so, were he discussing necrosis.

In classifying diseases by the processes affected, we were confronted right away with the usual headache of all classifiers. This is the problem of genus and species. Not all facts are of the same size. The big facts are made up of little facts and the big processes of plant life are comprised of a host of little processes.

We decided on six big processes; tissue is disintegrated; growth is



affected; reproduction is affected; host is starved; water is deficient; respiration is altered. A different pair of editors might have decided on a slightly different number of major processes. Some editors would doubtless elevate photosynthesis to chapter level. They would probably say, if you raise respiration to chapter level, why not photosynthesis? Translocation of sugars and other metabolites is surely a very important process in the plant that is subject to disruption by disease. Perhaps this should have been raised to chapter level, too.

One of the cardinal principles of information theory, however, is that all categories should be as nearly the same size as possible. We hoped that the six processes chosen would divide up the available information into approximately six equal amounts. Admittedly this was a matter of opinion, because no statistical information was available at the time.

We realized, even at the beginning of the work on design of the treatise, that information available on venereal diseases of plants was in small supply. Still we believed that the really fundamental importance of reproduction to life compels assigning chapter status to them, even though the chapter might work out a little thinner.

At any rate, the die is cast and the pathological processes are classified into six groups.

### VIII. THE RESISTANCE-SUSCEPTIBILITY PROBLEM

One of the classic divisions of plant pathology is usually termed resistance. This treatise contains no chapter by that name. Another classic division is susceptibility and we have assigned no chapter by that name.

We consider that health is the usual state of affairs. If the plant species does not remain reasonably healthy the species becomes extinct. Thus, the mere existence of a species proves that disease is abnormal and, hence, that susceptibility is abnormal.

On the other hand, the plant is almost continuously buffeted and battered by pathogens. In order to maintain itself and thus its species in existence, it must be able to fend off its enemies.

We are convinced that the plant fends off its enemies by dynamic processes as well as by static devices. The plant must expend energy to defend itself. It must expend some of its income on defense in the same way that an individual or a nation must expend income on defense.

We have avoided a chapter or chapters on resistance because we wanted to emphasize that defense is a dynamic state of affairs. Defense involves not only a chain link defense around the property. It must also involve constant patrolling by armed police.

Hence, we have used the major heading defense devices, and we have put three chapters under it. A chapter on histology discusses the physical type of barriers available as defense devices. The biochemistry of defense is one of the highly intriguing aspects of modern plant pathology. This has attracted many investigators but vastly fewer than its interest and significance warrant. The pickings in the field have been meager and some pathologists are discouraged—unwarrantedly so, we believe. This field and the field of chemotherapy will fertilize and cross-stimulate each other as the years go by.

Hypersensitivity is a chapter that generates some uncertainty. It is a subject with a large literature and, hence, warrants treatment. Probably much of its explanation will eventually come from studies of biochemistry of defense. We include it chiefly because of its novelty and because it epitomizes a kind of inverted defense that appeals to scientists.

#### IX. PREDISPOSITION

Predisposition attains chapter status because we believe it covers the essential activity of the ecologists in the field of plant pathology. Diseased plants are affected by their environment just as healthy plants are, perhaps more so. Plants growing in some environments put up less defense than in other environments and vice versa.

Plants are predisposed by their environment. Predisposition is somewhat difficult to treat because much of the experimental work is confounded. Very often the experimental environment is imposed at the time of inoculation so that the plant does not have time to become adjusted to it before the onslaught of the pathogen. Sometimes this type of experimental design gives very useful information, but the information is more useful if the plants are exposed for a time before inoculation as well.

And finally we come to therapy of the diseased plant. This is a method of control of disease in single plants. The usual control methods for disease are aimed at interdicting dispersal of pathogens from diseased to healthy plants. The pathogens are outside the host at the time.

Therapy is aimed at plants that are already infected.

Surgery is used to some degree in plant pathology under the old Biblical dictum "If thine eye offend thee, pluck it out." Its most useful application is in ornamental trees where the expense is offset by the aesthetic gains.

Chemotherapy is a new frontier which is slowly gaining ground. This is a most difficult problem, however. When the researches in the field of biochemical defense are married with those of chemotherapy, both should gain.

## REFERENCES

- Anonymous. 1950. Definitions of some terms used in plant pathology. *Brit. Mycol. Soc. Trans.* **33**: 154-160.
- Baldacci, E., and R. Ciferri. 1949. Saggio di una classificazione delle malattie delle piante su basi fisio- e morfoptatologiche. (An attempt to classify plant diseases on a physio- and morphopathological basis.) *Atti soc. ital. patol.* **1**: 59-64 (*Rev. Appl. Mycol.* **29**: 164, 1950).
- Berkeley, M. J. 1857. Vegetable pathology. Spread through *Gardeners' Chronicle* from 1854 to 1857.
- Brooks, F. T. 1928. "Plant Diseases." Oxford Univ. Press, London and New York. 386 pp.
- Chester, K. S. 1942. "The Nature and Prevention of Plant Diseases." Blakiston, Philadelphia. 584 pp.
- De Bary, A. 1866. Neue Untersuchungen über die Uredineen. *Monatsber. Königlich preuss. Akad. Wiss. Berlin* **1865**: 15-50.
- Duggar, B. M. 1909. "Fungous Diseases of Plants." Ginn & Co. New York. 508 pp.
- Duhamel du Monceau, H. L. 1728. Explication physique d'une maladie qui fait perir plusieurs plantes dans le Gatinois, particulièrement de safran. *Mem. Acad. Sci. Paris* **1728**: 100-112.
- Eriksson, J. 1930. "Fungous diseases of plants" (Wm. Goodwin, Trans.). Bailliere, Tindall, & Cox, London. 526 pp.
- Hallier, E. 1868. "Phytopathologie." Wilhelm Engelmann, Leipzig. 373 pp.
- Holmes, F. O. 1939. "Handbook of Phytopathogenic Viruses." Burgess, Minneapolis, Minnesota. 221 pp.
- Horsfall, J. G. 1956. The fight with the fungi or the rusts and the rots that rob us, the blasts and the blights that beset us. *Am. J. Botany* **43**: 522-536.
- Horsfall, J. G. 1959. A look to the future—the status of plant pathology in biology and agriculture. In "Plant Pathology, Problems and Progress, 1908-1958." American Phytopathological Society. Univ. Wisconsin Press, Madison, Wisconsin. In press.
- Link, G. K. K. 1933. Etiological phytopathology. *Phytopathology* **23**: 843-862.
- Morstatt, H. 1929. Pflanzenpathologie als Wissenschaft und Unterrichtsgegenstand. *Proc. Intern. Congr. Plant. Sci., 1st Congr. Ithaca, 1926* **2**: 1194-1203.
- Plenck, J. J. 1794. Physiologia et Pathologia Plantarum. Viennae. (Cited by Hallier, 1868.)
- Reddick, D., N. E. Stevens, and J. I. Wood. 1940. Report of the committee on technical words. *Phytopathology* **30**: 361-368.
- Sorauer, P. 1886. "Handbuch der Pflanzenkrankheiten," 2 Vols. Paul Parey, Berlin.
- Stakman, E. C., and J. C. Harrar. 1957. "Principles of Plant Pathology." Ronald Press, New York. 581 pp.
- Stevens, F. L. 1917. Problems of plant pathology. *Botan. Gaz.* **63**: 297-306.
- Thaxter, R. 1890. Report of the mycologist. *Conn. Agr. Expt. Sta. (New Haven) Rept.* **1889**: 127.
- Unger, F. 1833. "Die Exantheme der Pflanzen." Carl Gerold, Vienna. 422 pp.
- Walker, J. C. 1950. "Plant Pathology." McGraw-Hill, New York. 699 pp.
- Walker, J. C. 1957. "Plant Pathology." McGraw-Hill, New York. 707 pp.
- Ward, H. 1901. "Disease in Plants." Macmillan, London. 309 pp.
- Whetzel, H. H. 1918. "An Outline of the History of Phytopathology." Saunders, Philadelphia. 130 pp.

- Whetzel, H. H. 1928. The relation of plant pathology to human affairs. In "Mayo Foundation Lectures." Saunders, Philadelphia. pp. 151-178.
- Whetzel, H. H. 1929. The terminology of plant pathology. *Proc. Intern. Congr. Plant Sci. Ist. Congr., Ithaca*, 1926 2: 1204-1215.
- Zallinger, J. B. 1773. "De morbis plantarum cognoscendis et curandis dissertatio ex phaenomenis deducta." Oemponti. 137 pp. (Cited by Whetzel, 1918.)





## CHAPTER 2

# Scope and Contributions of Plant Pathology

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## I. INTRODUCTION

Plant pathology sweeps across most of the sciences in botany. It dips into zoology when animals cause disease. It must deal with soils, meteorology, physics, chemistry. This is precisely the same situation as prevails in medicine and veterinary science.

The chemist must deal with a wide range of science, but it is hardly to be compared with the wide ranging scope of plant pathology.

In this chapter we hope to suggest how the other sciences impinge on plant pathology and how it impinges on some of them. Also the importance of plant pathology for several aspects of human life will be discussed.

## II. PLANT PATHOLOGY AMONG THE SCIENCES

### A. *Scope of Plant Pathology*

For a good understanding of the scope of plant pathology, we must go back to the middle of the 19th century, when this science began. At that time, the theory of spontaneous generation of microbes and fungi was still accepted by many scientists. Botany, including plant taxonomy, physiology, and mycology, was gradually evolving from the merely descriptive stage into the more dynamic one of experimentation. In Germany, old erroneous ideas about the causes of parasitic plant diseases began to tumble down mainly as a result of the excellent work of the brilliant young mycologist Anton De Bary and the critical writings of Julius Kühn, who published the first book dedicated to plant pathology as a whole in 1858. In 1853, De Bary wrote his first paper on plant diseases proving by experiment, that smuts and rusts of cereals are due to pathogenic fungi. He showed that the fungi are not exudates of diseased tissue and that they are not spontaneously generated. This was quite revolutionary, as at that time leading scientists believed that fungi on diseased plants derived from waste material of the plant whereas diseases were thought to be due to a general inner disease predisposition present in all crop plants but absent in wild species. Thus arose experimental plant pathology.

As the science of plant pathology partly evolved from plant physiology it is not surprising that all abnormal growth of plants belonged to its field of study, independent of the primary cause of the abnormalities. Plant galls due to mite infestation or deformations resulting from the presence of insects, such as gall midges, were just as important to the first pathologists as diseases due to fungus attack or unfavorable weather conditions such as heavy frost or abnormal drought.

Probably the early development of the science of plant pathology in Germany is responsible for the fact that descriptions of damage due to insect pests still are found in German handbooks such as the well known six-volume "Handbuch der Pflanzenkrankheiten."

Many disturbed physiological processes of the plant result from adverse environmental conditions either in the soil (e.g., mineral deficiencies or excess of certain elements, lack or excess of water) or in the atmosphere (e.g., too low or too high temperatures, presence of poisonous industrial gases).

In many other cases a living pathogen is responsible for the disease. Fungi were the first living pathogens to be recognized. Bacteria, the most dangerous germs for men, were discovered much later as plant pathogens. Although they may cause severe diseases in plants especially when the climate is hot during the growing season, the number of diseases caused by fungi is far greater. More recently, viruses were recognized as the cause of many diseases, and also eelworms (nematodes) were found to cause serious damage to crops. The importance of the latter group is gradually being recognized. Within the near future nematology will probably develop to such an extent that it will be impossible to treat this subject in a handbook like the present one. Of course, specialization is necessary but one should never forget that the disorders in plant life are complicated physiological phenomena in which more often than not several organisms and the environment play an important role.

In the following paragraphs an attempt will be made to discuss those disciplines which impinge on plant pathology and on which plant pathology impinges.

## *B. Relation between Plant Pathology and Other Sciences*

### *1. Physiology of Healthy and Diseased Plants*

Plant physiology is the science most closely related to plant pathology. A disease has been defined as an abnormal physiological process. Hence, plant pathology is concerned with abnormal physiology.

Plant physiology has contributed greatly to plant pathology. Plant pathology makes use of essentially all that the plant physiologist has learned. This may be illustrated with some examples.

Nutrition is basic to the life of plants as well as of animals. A shortage of trace elements such as manganese, zinc, and boron induces disease symptoms and application of the right fertilizers may reverse the process and bring the plants back to normal. Thus, it has been

possible to cultivate new lands by adding trace elements and phosphates, e.g., in southern Australia, which hitherto were too low in fertility to allow a profitable crop production.

Photosynthesis and the distribution of assimilation products influence and are influenced by pathogenic infections as shown in more detail in Chapter 8 of this volume. Recent investigations suggest an increase in photosynthesis during the first few days after infection (Sempio, 1950) and a subsequent decrease especially in heavily infected plants (Allen, 1942). In the earlier literature, too little attention was paid to the trend in photosynthesis with time, some investigators measuring photosynthesis at an early stage of infection whereas others used plants with an established infection (Yarwood, 1957). However, photosynthesis always decreases within a few days after infection.

The chemical constitution of healthy leaves changes during the growing period and simultaneously the susceptibility for diseases alters. For example, only young apple leaves of a certain age are attacked by *Venturia*. The older leaves remain free from the disease even after artificial inoculation, and according to Grümmer (1955) there is a correlation between the infectivity of potato leaves by the late blight fungus *Phytophthora infestans* and protein decomposition in the leaf tissues. This decomposition takes place as a result of initial tuber formation. This fascinating subject is treated in Chapter 12 of this volume.

Many diseases increase transpiration in plants, often in combination with an increase in permeability (Thatcher, 1939). Whether this increase in permeability is responsible for the mobilization of substances from adjacent healthy tissue or whether such a mobilization is due to metabolic changes is not quite clear.

Pathological wilting may occur, e.g., as a result of the action of vascular parasites attacking the roots and lower parts of the plant. These parasites (fungi or bacteria) may produce toxins which according to Gäumann (1951) have a coagulating effect on the protoplasm of the host cells resulting in water release, and an excessive rate of transpiration. In Section 8, we shall see that other workers hold another opinion, with respect to the biochemical background of wilting.

Not only does the plant pathologist depend on the plant physiologist for a better understanding of the pathological processes going on in a diseased plant, the physiologist may also gain understanding of normal physiological phenomena by using "abnormal" (i.e., diseased) plants (Suchorukov, 1957). Thus, Bennett (1937) studying curly top (a virus disease) of sugar beets, contributed substantially to our general knowledge about translocation of food in plants. Another example is dormancy.

Braun cites an interesting effect of witches' broom on dormancy (see Chapter 6 of this volume). The elucidation of this effect should be valuable in normal plant physiology. But unfortunately such reciprocal features of plant pathology are often ignored by the physiologist.

## 2. *Pathological Anatomy and Morphology*

The histologists, anatomists, and morphologists have contributed their share to plant pathology. Various histological and morphological characters of the plant influence and are influenced by disease. These matters are subject to detailed analysis in Chapter 11 of this volume and in Chapter 6 of Volume II. A few examples suffice to outline the subject for our purposes here. Many infections induce local necrosis of tissue, e.g., in the case of hypersensitivity of a host for a pathogen (see Chapter 13). The pathogen dies when the adjacent cells are induced to divide forming a protective meristem, often consisting of corky cells (periderm). Potato leaves become brittle in case of leaf roll infection. This virus disease produces typical changes in the cells of the phloem that prevent a normal downward stream of assimilation products.

Several instances of pathological plant growth and monstrosities are mentioned in the Report of a Symposium on Abnormal and Pathological Plant Growth (1953) including tumors due to the crown gall bacterium (*Agrobacterium tumefaciens*) and to virus infection. The former will be treated more in detail in Chapter 6 of this volume. Abnormalities range from the formation of "giant cells" (e.g., caused in potato roots by *Heterodera rostochiensis*, the potato root eelworm) to leaf enations, green protrusions mostly on the underside of the leaves, as in the case of rasp leaf virus disease of cherries, and cup-like outgrowth of the veins, being a symptom of the so-called "kroepoek" virus disease of tobacco (Kerling, 1933).

Witches' brooms are also common disease symptoms that arise from the forcing of dormant buds. Rubus stunt virus of raspberries (Prentice, 1950) induces phyllody which means a change of floral organs (sepals, petals, carpels, and stamens) into leafy structures; they are sometimes of exactly the same shape as normal leaves, e.g., with clover. Strangely enough in many cases the stamens are not altered. A recent survey of the literature on this subject is given by Bos (1957). Although these peculiar pathological forms have been known for a long time, it is only recently that viruses have been found to be the causal agents in many cases. This type of disease is not just a teratological rarity, but it may spread epidemically causing severe losses to the growers (Rhind *et al.*, 1937; de Fluiter and van der Meer, 1953).



Here also we find reciprocal contributions of plant pathology. The crown gall disease has contributed greatly to the knowledge of differentiation. It has also been valuable to students of human cancer. Floral morphology was elucidated by the study of phyllody.

### 3. *Genetics, the Basis for Breeding Resistant Varieties*

Little as we may know of the physicochemical processes that prevent a pathogen from establishing itself in plant tissues, there are nevertheless many examples of successful breeding of disease resistant varieties of our agricultural and horticultural crops. Modern plant breeders have provided us with an increasing number of valuable crop plants that are resistant to some or all the major diseases. Stakman treats these problems in Chapter 14 of Volume III.

Unless the etiology of the disease-causing agent is known, the chances of obtaining resistant varieties are limited. Admittedly, some very successful resistant varieties have been produced without knowing anything of the causal agent of the disease. One of the classic examples is sugar cane resistance to the so-called sereh disease on Java. Much later it was found that sereh disease is caused by a virus.

Originally, breeders used only mass selection. They picked out the healthy plants in a heavily infested population and there was some risk that such plants had escaped infection. Subsequent testing demonstrated whether or not a resistant strain had been obtained. An early example of this procedure is the success of L. R. Jones (1914) in selecting yellows resistant cabbages.

Mass selection is less promising than formerly, however, because our agricultural and horticultural crop varieties are usually more homogeneous than before and comprise fewer biotypes than the old indigenous breeds of crops.

Because of this, the potential danger of a serious outbreak of disease is greater today than it used to be. Plant breeders are well aware of this menace.

The rediscovery of Mendel's laws of heredity in 1901 greatly facilitated the use of genetics in plant pathology.

Biffen (1907), during his studies on wheat genetics at Cambridge, was the first to discover that resistance and susceptibility to yellow rust (*Puccinia glumarum*) are inherited characters following the same rules as the inheritance of morphological characters. When his rust resistant varieties turned out to be susceptible in Australia there was much disbelief in genetically bound disease resistance. However, with the discovery of physiological races of the pathogen, Biffen's conclusion was generally accepted.

Often, the species or variety carrying the resistant gene is of no value as a crop plant. One must then make several back crosses to the valuable but susceptible cultivated variety, thus combining the genes for resistance with valuable market qualities. Such a procedure is a time consuming business. A period of 10 years is quite normal before an acceptable new variety is obtained.

In order to prevent disappointments, a knowledge of the disease and its causal agent is imperative in deciding on the inoculation methods, and the best ways of establishing a local epidemic (Coons, 1953). The breeder should be aware of the great variations in pathogenicity within the races of the pathogen (fungus, bacterium, or nematode). The study of the genetics of pathogens, therefore, is of the greatest importance for breeder and plant pathologist alike. While the geneticist or mycologist may study the inheritance of any given property of a pathogen such as color, spore morphology, or biochemical reactions (for details see Beadle, 1945, and Catcheside, 1951), the plant pathologist is especially interested in its pathogenicity to the host plant.

A complication in the plant pathologist's work is the fact that the races of the pathogen seldom differ morphologically but only in virulence toward the host plant. Therefore, these races can only be distinguished by their behavior toward different varieties of the host plant. For many diseases such differential varieties are being used with success, e.g., in the case of stem rust of wheat (Stakman *et al.*, 1944), rust of flax (Flor, 1954), anthracnose of beans (Andrus and Wade, 1942; Hubbeling, 1957). More details on this subject will be given in Volume III, Chapter 14.

Plant pathology has contributed to genetics with studies on the sexual reproduction of pathogenic fungi and on the genetic background of pathogenicity (e.g., Keitt *et al.*, 1943; Keitt and Boone, 1954: *Venturia inaequalis*; Flor, 1955: flax rust). As an example of the complications met with in this work, the outstanding contribution of Flor (1955) may be mentioned. He explains host-pathogen interaction in flax rust by assuming a gene-for-gene relationship between rust reaction in the host and pathogenicity in the parasite. In flax and the flax rust fungus Flor has been able to identify 25 such pairs of genes.

Comparable results have been obtained by Black *et al.* (1953) using races of *Phytophthora infestans* on potato.

How the so-called Fungi imperfecti form physiological races has long been obscure. It was mostly thought to be due to mutation, but recent work of Pontecorvo *et al.* (1953; Pontecorvo and Sermoniti, 1954) and Buxton (1956) throws some new light on this phenomenon. It seems that heterokaryosis as found by Buxton for *Fusarium oxysporum* f. *pisi* (a cause of pea wilt) is not uncommon and it is not at all improbable

that a parasexual system as described by Pontecorvo and Sermonti (1954) is responsible for the formation of new physiological races in many of the Fungi imperfecti. On culture media, anastomoses between adjacent hyphae of different strains of a fungus are quite common and there is no reason why this should not occur in nature.

From the foregoing, the fundamental mutual importance of genetics and plant pathology will be clear. Both disciplines should be obligatory in the training of students in plant pathology. This is already the case in almost every university in the United States. The important role that American plant pathologists play in the development of so many disease resistant varieties of crop plants in the United States may be due to the fundamental knowledge of genetics present with the student in plant pathology in that country.

#### *4. Plant Taxonomy and Plant Geography*

Plant geography and taxonomy have also contributed to the science of plant pathology. Vavilow (1935, English translation 1949-50) first realized that cultivated crops had probably lost important genes and that these could be recovered only by collecting taxonomically related species and genera from the centers of origin of our crops. In such work, expertness in taxonomy and plant geography is important.

Vavilow and others traveled to all eleven regional centers of origin of our crops throughout the world and collected more than 300,000 samples of seed and seedling material. Enormous unknown varietal resources even of such crops as wheat, potato, corn, legumes, rye, and flax were discovered. As Vavilow says: "In the case of certain plants such as the potato, the newly discovered species and varieties literally revolutionized our conception of the source materials."

Careful plant geographic studies had to precede the expeditions and finally the large collections had to be studied taxonomically. Vavilow concludes his chapter on the phytogeographic basis of plant breeding as follows: "The enormous plant potentials discovered in the centres of primary origin of forms and species of cultivated plants, should be subjected to investigation not only by the taxonomist, but also by the physiologist, the biochemist, and the pathologist. In the field of genetics, which aims at new creations through the most rational combinations of parents, an immense field of the most fascinating and urgent work is opened up."

Many of the species collected by the Russians are of an endemic type. This means that their biochemical constitution may be entirely different from that of the commonly used cultivated varieties, thus providing a new base for resistance to pests and diseases to which they

have not been exposed in their original habitat. This so-called preadaptation resistance seems of great importance (Harland, 1955).

It is not surprising that Vavilov's publications aroused much interest all over the world. American, German, and other expeditions were sent to the promising gene centers. Large collections of as many varieties, related species, and genera as could be found, were planted in experimental plots to be used as a gene reservoir. In order to prevent introduction of dangerous new diseases or pests, such plants had to stay in quarantine before they were released to the breeding stations. There these stocks are being tested for resistance to the major diseases. The interregional potato introduction and preservation project at Sturgeon Bay, Wisconsin, for instance, is testing the reactions of all collected material to *Verticillium* wilt, *Fusarium* spp., common scab, late blight, early blight, Southern bacterial wilt (brown rot), ring rot, black leg, virus A and X, and leaf roll (Stevenson and Akeley, 1953). Similar work is done at the Max Planck Institute for Plant Breeding at Köln/Vogelsang, Germany, where immunity from viruses X, B (closely related to X), Y, A, and leaf roll was found in a number of wild potato species (Ross and Baerecke, 1950).

### 5. *Mycology, One of the Important Foundations of Plant Pathology*

Mycology is the science that fathered plant pathology. That is because fungi are bigger than bacteria and viruses and could be seen first with primitive microscopes. Plant pathology could not become a science as long as etiology was lost in a haze of spontaneous generation. Originally, mycology was a purely descriptive science devoted to the classification of fungi. One of the outstanding early mycologists was E. M. Fries, of Sweden, who published his "Systema Mycologicum" over the years 1821–29. This work forms the basis for systematic mycology in much the same way as Linnaeus' "Species Plantarum" became the classic for plant taxonomy. However, Fries' ideas on plant parasitic fungi, which he brought together in a section called Hypodermii, because they were found under the skin of plants, were fully in accordance with the opinion of his contemporaries. He stated: "These fungi depend on a diseased condition of the plant rather than vice versa. From the study of many examples, we have learned that they are hereditary and that they depend on the composition of the atmosphere. Every fear of their propagation by sporidia is superfluous" (cited by Brown, 1951).

The idea of the independent existence of microscopic fungi became generally accepted only after the appearance of the epoch-making publications of the Tulasne brothers (1861–65) in France and of De Bary (1853, 1866) in Germany.



The development of synthetic sterile media for the cultivation of fungi was a seven-league step for mycology and plant pathology. It converted etiology to a scientific reality. It enabled an ever increasing number of scientists to study the physiology, sexuality, sporulation, nutritional wants, and genetics of countless saprophytic and parasitic fungi.

In the meantime, systematic mycologists went on describing new species compiled in Saccardo's well known "Sylloge Fungorum" (1882-1931), now containing no less than 80,000 names! After the beginning of the 20th century, it became evident that the species limits for many of these fungi were too narrow since most of them had been described without taking into account the influence of environmental conditions on morphology. Each species contains many strains, and the range of characters shown by the sum of strains is wider than that of the original strain. For us as plant pathologists, physiological strains are of even greater importance than morphological ones as the virulence of one strain may differ greatly from that of another, e.g., with the rust fungi on cereals.

A special group of mycologists study those fungi living symbiotically in the roots of trees where they give rise to morphological changes called mycorrhiza. It is interesting that Basidiomycetes, are particularly important in this role. They include toadstools, such as *Boletus* and *Amanita*. Their role seems to be very specific, one *Boletus* species being associated only with one type of tree.

#### 6. *Virology, a Study of the Borderline between Living and Dead Substances*

MOTTO: *Nature makes so gradual a transition from the inanimate to the animate kingdom, that the boundary lines which separate them are indistinct and doubtful* (Aristotle, cited from K. M. Smith, "Beyond the Microscope").

After many centuries of disbelief, this 2000-year-old remark of the famous disciple of Plato is gradually being accepted by more and more scientists.

Without going into further detail on this philosophical concept, one may say that only viruses possess characteristics partly specific for living organisms and partly inherent in dead substances. Mycology may have whelped plant pathology, but plant pathology has done much toward developing the science of virology.

The first virus to be isolated in a para-crystalline form was the tobacco mosaic virus (Stanley, 1935). This accomplishment eventually



won a Nobel Prize for Stanley. Even to this day the tobacco mosaic virus continues to contribute importantly to knowledge of virus and even to knowledge of genes and their function.

Virology has very rapidly grown into a separate discipline because viruses of man, plants, livestock, insects, and bacteria have many properties in common. Results obtained in one group are as a rule of great interest to the virus workers in the other specialties.

Isolation of the virus in as pure a form as possible is essential for further studies. Only recently through the use of the ultracentrifuge, electrophoresis, chromatography, and electron microscope, a number of viruses have been obtained in a relatively pure state. This engenders admiration for Stanley (1935) and his co-workers, who had to crystallize tobacco mosaic virus by using ordinary chemical methods. The tobacco mosaic virus now appears to be a nucleoprotein with a molecular weight of about 40,000,000. It has been possible to measure the size of the virus particles by ultrafiltration or X-ray application. Later, these data were confirmed when the virus particles were visible with an electron microscope at a magnification of 10,000 to 30,000 times.

In order to understand fully the biological activities of a virus, it is desirable to know the special configuration of the virus molecule. This seems at the moment still a superhuman task (Thung, 1957). Nevertheless, recently considerable progress has been made in this field (Perutz, 1958).

One of the general virus problems is the attachment to and penetration of cell walls by viruses. Here, the workers with bacteriophages of the T system have got the lead (Tolmach, 1957).

Another and perhaps the most important general problem is that of virus multiplication in the host cells. This will be considered in detail in Chapter 3 of Volume II.

Animals develop resistance to viruses by producing antibodies in the blood. Since plants do not form antibodies, the methods used with animals to stimulate antibody formation are useless in treating plant virus diseases. However, for diagnostic purposes, serology is used on a large scale in plant virus work. Rabbits and other animals are used for the production of antibodies by injecting the purified plant virus into their veins. A whole range of antisera have been prepared in this way, and these are used by the General Netherlands Inspection Service for Seeds of Field Crops and for Seed Potatoes (N.A.K.) for the production of healthy seed potatoes free from viruses X, S, and M. The antisera are prepared by the Laboratory for Bulb Research at Lisse, where the mass-testing techniques also have been developed (van Slogteren,

1955). It is interesting to note that the presence of potato virus S was discovered during serological research at Lisse. This virus may cause 15% yield reduction and now that it has been discovered, it seems to be widespread in Europe and the United States.

#### 7. *Applied Entomology, an Indispensable Help to the Plant Pathologist*

During succeeding decades, plant pathology began to neglect insects as pathogenic organisms and drifted into mycology, bacteriology, and virology. The role of insects as causes of plant diseases and injuries was left to entomologists who founded the science of applied entomology.

Plant pathologists have, however, also contributed to applied entomology by their studies of symbiosis between insects and microorganisms.

A typical example of a mutualistic symbiosis is the case of bacterial soft rot affecting many vegetable crops. This disease is spread by the seed-corn maggot, *Hylemyia cilicrura* (Rond.) and other dipterous insects. The maggots can develop normally only in plant tissues decayed by the bacteria. The soft rot bacteria, in turn, cannot penetrate uninjured plant tissues, but grow abundantly once the plants have been wounded, e.g., by maggots (Leach, 1952). In this respect, it is interesting to note that the bacteria survive within the intestinal tract of the insect in all stages of metamorphosis including the eggs.

Much more common is the spread of diseases by insects which apparently do not benefit from this transmission at all but merely act as vectors. The first report of such an insect transmitted plant disease was given by Waite (1891), who proved that the bacteria causing fire blight of pears are transported by bees and wasps while visiting blossoms in search of nectar. This subject is treated in considerable detail by Broadbent in Chapter 4 of Volume III.

The Japanese entomologist Takami discovered as early as 1901 that the dwarf disease of rice in that country developed as a result of the feeding of the leaf hopper, *Nephotettix apicalis* Uhl. He did not know, however, that this leaf hopper acted as a vector of an infectious disease. This was established by other Japanese workers in 1908. It was many more years before the virus nature of the disease could be proved.

If one realizes how long ago leaf hoppers have been indicated as vectors of virus diseases in Japan, United States, and Russia, it is surprising that no leaf hopper transmitted virus disease has been reported from Western Europe before 1953 when de Fluiter and van der Meer (1953) found the leaf hopper *Macropsis fuscula* Zett. to be the vector of rubus stunt, a virus disease of raspberries. This may be due to the fact that the leaf hoppers are more frequently vectors of virus diseases, in subtropical and tropical climates than in cooler climates.

There is another interesting problem in relation to the transmission of virus diseases. Why are some viruses nonpersistent (allowing the insect carrier to transmit the pathogen soon after feeding on a diseased plant) whereas with others—the persistent viruses—an interval of up to several weeks may be necessary before the insects can infect healthy plants? The latter case seems to be the rule with viruses transmitted by leaf hoppers. Here apparently the virus multiplies in the insect body as the insects remain infective through several successive generations (Black, 1950; Black and Brakke, 1952). Other instances are reported where the nymph of a leaf hopper can pick up a virus but is not able to transmit it until the adult stage is reached. This also points in the direction of virus multiplication in the insect body. Still different is the case of a thrips (*Frankliniella insularis*), transmitting spotted wilt virus of tomato. Here, the adults become infective only after the larvae have sucked on a plant diseased by virus (Bald and Samuel, 1931).

## 8. Biochemistry and Plant Diseases

Biochemistry is a rapidly rising science which is contributing very fundamentally to our knowledge of the abnormal processes in disease.

In many ways the advances in science are derived from advances in technique. Biochemical techniques are developing so fast that they almost tumble over each other's heels and every new technique is soon reflected in advances in plant pathology.

The more that is known about the biochemical processes taking place at the foci of infection the more it becomes clear how complicated pathogenesis is. This fascinating and most fundamental section of plant pathology is getting more and more attention from the modern research worker. The difficulties he faces are tremendous, but important achievements attained thus far have stimulated yet more research aimed at a better understanding of pathogenesis.

The possible role of toxins in the development of disease symptoms is a typical biochemical problem. This is especially the case with wilt diseases. Many plant pathologists still adhere to the theory that toxins induce the wilt diseases (Gäumann, 1954). Others however, have recently questioned the role that toxins such as lycomarasmin, vasinfuscarin, and fusaric acid play in the syndrome of wilt diseases, etc., because such phytotoxic compounds isolated from culture filtrates of a pathogen have not been detected in or isolated from diseased plants (Dimond, 1955). A possible exception is ethylene. The epinasty and leaf-yellowing symptoms of wilt diseases seem to result from the action of ethylene formed by the pathogen and possibly to some extent by the host.

It would, therefore, be wrong to regard all toxin formation of pathogenic fungi and bacteria as unimportant in pathogenesis. Wildfire disease of tobacco caused by the bacterium *Pseudomonas tabaci* may serve as another example. The biochemistry of this disease has been studied in detail. It produces localized chlorotic halos surrounding central brown necrotic leaf spots. In this case, a characteristic toxin has been isolated. It is a structural analogue of methionine, one of the amino acids essential for plant growth.

The wildfire toxin owes its biological activity in one and perhaps in all susceptible plant species to its antimetabolite properties, i.e., the toxin molecule is able to replace the structurally related essential metabolite, thus causing the development of pathological symptoms (Braun, 1955). Toxins are discussed in much more detail in Chapter 9 of Volume II.

Much biochemical work has been done with the aim of elucidating the cause of disease resistance. Biochemists have, in the first place, tried to isolate chemicals which were only present or occurred in a much higher concentration in the nonsusceptible than in the susceptible variety. Classic examples of such chemicals are protocatechuic acid and catechol isolated from the dry outer scales of pigmented onions (Link and Walker, 1933). Such onions are highly resistant to *Colletotrichum circinans* (Berk.) Vogl. and several other pathogens. Resistance depends on the presence of the dry outer scales, as here the toxic substances are easily accessible in a water soluble form and prevent germination of the fungus spores.

The biochemistry of enzyme activity has also advanced our knowledge of pathogenicity.

Thiourea, 2,4-dinitrophenol, and sodium fluoride break down resistance in several plant species. Substances having this effect are all inhibitors of the activity of respiratory enzymes. These enzymes seem to play an important role in disease resistance (Walker and Stahmann, 1955; Fuchs and Kotte, 1954; Hassebrauk and Kaul, 1957). It has been found for instance that the activity of the ascorbic acid oxidase system was higher in wheat plants resistant to brown rust (*Puccinia tritricina*) than in susceptible wheat varieties (Hassebrauk and Kaul, 1957). In wheat affected by stem rust (*Puccinia graminis tritici*) the activity of other enzymes (e.g., glycolic acid dehydrase and glutaminic acid decarboxylase) was much reduced. Since at the same time ascorbic acid oxidase is stimulated the normal enzymatic balance of the healthy plant is shifted to new metabolic chains in the diseased plant (Farkas, 1957).

In this respect it is interesting that hypersensitive reactions occurring in resistant varieties of various crops have been attributed to nonspecific



“defense bodies” which are formed some hours after infection of the host cell has taken place. The name phytoalexin has been proposed for these substances (Müller, 1957). They can be isolated from the host tissues and are toxic to several unrelated pathogenic fungi. The fact, stressed by Müller, that they are formed only after infection has taken place seems of particular importance, also in connection with the statements made by several investigators that fungitoxic materials isolated from healthy noninfected plants may not be responsible for resistance. The apparently easy transport of many chemicals to the site of infection as described by Shaw and Samborski (1956) may be better understood in the light of recent cytophysiological and cytochemical studies which led to considerable doubt on the presence of a membrane of high resistance outside the cytoplasm of the cell. Instead, the theory of apparent “free space” has been put forward. According to this concept, the cytoplasm has water-filled spaces continuous with the water-filled spaces of the cell wall and the intercellular space systems allowing free diffusion between the aqueous phases of the cytoplasm and an external solution, e.g., of intermediates of metabolism (Robertson, 1957).

In the case of plant virus diseases, the great stimulus to biochemical research was given by Stanley's isolation of tobacco mosaic virus (Stanley, 1935). The following years revealed that several plant viruses, including tobacco mosaic virus (TMV) consist of ribonucleoproteins (Bawden and Pirie, 1937, 1938; Stanley and Knight, 1941). In fact, up till now, no plant virus has been found which does not contain proteins and nucleic acids (Markham, 1953).

Modern isolation techniques such as chromatography and electrophoresis enabled research workers to separate the amino acids of the proteins and the various constituents of the nucleic acids. Recent investigations, both with respect to the amount of purines and pyrimidines present in the nucleic acid part of the virus and analysis of the amino acid composition of the proteins, revealed marked differences between various viruses (Schramm and Keréjártó, 1952; Black and Knight, 1953).

Much more biochemical work must be done in relation to pathogenesis and for diagnostic purposes. We can see only the beginning.

#### 9. *Chemistry, One of the Pillars of Modern Plant Protection*

That the title of this paragraph is not an overestimation of the important role chemistry plays today in plant protection may be illustrated by some examples. Seed treatments with fungicides, now being a common practice, e.g., with cereals and vegetable crops, is the cheapest insurance the farmer can take against certain plant diseases, especially



those caused by "damping-off" fungi. Before seed treatment came into use, many crops had to be replanted at considerable cost. Treatment of spinach seed at a cost of only 30 cents yielded a \$300 gross return according to McNew *et al.* (1951). New very potent seed protectants such as thiram and captan have been added to organic mercury and inorganic copper compounds which were the favorites before World War II. -

Recently, new antibiotics (e.g., rimocidine) have been isolated from certain *Streptomyces* species. They have shown a systemic action, penetrating through the seed skin thus killing internal pathogens such as *Ascochyta pisi* in peas (Dekker, 1957).

In the United States, the yield of potatoes per acre doubled from 1939 to 1952 as a result of combined spraying of the foliage with DDT and ethylene bisdithiocarbamates (Dimond and Horsfall, 1955).

Control of soil-borne fungus diseases, other than damping-off, is still difficult to obtain, although some new approaches (e.g., with sodium *N*-methylthiocarbamate) look rather promising. Soil treatments with nematicides such as DD have given excellent results against plant parasitic nematodes.

As the potential market for pesticides is enormous, the great interest in plant protection shown by chemical companies is not surprising. More than 25,000 organic compounds have been tested as possible fungicides, and this number is still increasing daily. Less than 0.1% ever reaches the stage of practical application (Dunegan and Doolittle, 1953). The development of such a promising compound needs large investments ranging from \$250,000 to over \$1,000,000. Still, the prospects seem to be attractive. In the last 3 years, the average annual production of copper sulfate for fungicidal use amounted to 145,000,000 lb. in the United States. Total United States exports of pesticides increased to a value of \$85,909,000 in 1957 (Shepard, 1958). For the control of Sigatoka disease of bananas in Central America more than 45,000,000 lb. of copper compounds are applied each year! In fact, crop protection forms the highest cost in present day banana production.

As it is clear that it would be impossible to test every chemical compound in field trials, research must primarily be carried out by chemists and plant pathologists in the laboratory. Modern industrial research laboratories have been built for this purpose.

While the mode of action of fungicides is not yet fully understood, the search for new active chemicals will be more or less on a "trial and error" basis. Clearly, the ideal would be to predict the fungitoxic activity from the structure of a chemical compound just as the modern chemist working with plastics can now design new compounds with the desired

properties at his desk, and then prepare them in the laboratory. With the "trial and error" method we do not mean, however, that one should take all the available chemicals from the shelf and just screen them on their biological activity. As van der Kerk (1956) said: "Rather has one to admit that discoveries of new fungicides usually are based on knowledge also of distant domains of the natural sciences, on a keen intuition, and on the ability to make cross-links between apparently unrelated fields." As an example, he mentions the development of the dialkyldithiocarbamates as fungicides.

Gradually, a stage of knowledge is reached where one scientist of genius, capable of surveying the whole subject and finding time for constructive imaginative thinking, may detect the still lacking fundamental natural law lying at the very base of the most divergent physico-chemical interactions of the living organisms and their inanimate environment.

We now must face the practical implications connected with the use of fungicides. The laboratory techniques necessary for the development of new fungicides will be treated later (Chapter 14, Volume II).

Clearly, in plant disease control it is not sufficient to find a chemical with strong fungicidal activity. The product also should not be harmful to crop plants; moreover, it must be rain resistant. This sounds simple, but disappointments have been the reward of industrial chemists because in the field, some products have behaved differently from laboratory and greenhouse tests, both on phytotoxicity and on rain resistance.

The final product ready for marketing does not contain only the active ingredient and some inert material, but as a rule stickers and/or spreaders (wettters), and stabilizers are also added. This so-called "formulation" of the product, done by special chemists, is more an art than a science although gradually out of experience and knowledge some general principles are evolving.

Both for insecticides and fungicides there is at present a certain trend toward biocides which kill the noxious organisms but save the beneficial ones. An example is the selective action of thiram used as a soil fungicide, in which case the antagonistic fungus *Trichoderma viride* was not affected (Richardson, 1954). The most specific fungicide known at present is hexachlorobenzene, discovered in France for wheat bunt (*Tilletia caries*). In general, the organic fungicides seem to be more specific than the inorganic ones (Lilly and Barnett, 1951).

The chemist who develops new fungicides may have to deal with resistance of a fungus against the fungicide. Spraying of insecticides has frequently resulted in the development of resistant strains of the pest, and the use of antibiotics both in medicine and agriculture has led to

acquired resistance of pathogenic bacteria. Until recently, there was not much evidence that the same occurred with fungi, but Horsfall (1956, p. 96) gives some literature citations which undeniably demonstrate the possibility of the development of resistance of certain fungi with respect to such fungicides as copper compounds, organic mercury compounds, thiram, and tetrachloronitrobenzene. Recent German work on the differences in resistance between various strains of wood attacking fungi toward wood preservatives points in the same direction (Gersonde, 1958; Schulz, 1957).

Undoubtedly, the ideal chemical treatment of crops against fungus attack is chemotherapy, also called internal therapy, contrary to the conventional method, where the chemical is used as a protectant. In the case of chemotherapy, the fungus is killed after it has penetrated the plant tissue. Organic mercury compounds, for instance, can be used as therapeutants (eradicants) up until several days after infection of apple scab has taken place. Many promising laboratory and greenhouse experiments have been carried out with chemotherapy, but so far only a few practical applications of this method are known. Chemotherapy also includes the use of antibiotics, e.g., as seed dressings, and the control of deficiency diseases. In the latter case good results have been obtained by the application of the deficient trace element either to the soil or as a foliage spray. Chemotherapy will be treated in detail in Chapter 15 of this volume.

#### 10. *Technology Comes to the Rescue*

Much of the art of plant pathology requires engineering technology and the engineers have contributed their share. This is particularly true in the technology of spraying and dusting.

Bordeaux mixture was first applied to plants by sprinkling with a brush. This is not a very economical way of engineering. Gradually, mechanical sprayers were developed, beginning with knapsacks and passing on to larger power equipment. Originally it was thought essential to go on with spraying until "run-off," but doing so one usually wastes 95% of the liquid and even then one does not completely wet the plant (Fraser, 1957). This so-called high volume spraying, originally carried out with hydraulic sprayers reached its limit in the powerful speed-sprayers with air-propellers, carrying an arc of nozzles and discharging as much as 100 gallons/min. at a pressure of 55 lb./sq. inch. Only by making use of air, was it possible to let the liquid pass through a rather wide orifice, thus avoiding clogging and providing sufficient penetration. Although such a machine functions excellently (the fan making 2100 revs./min.) and although it can be operated by one man, the drawback

remains that one or more large tank wagons have to ride to and fro for filling purposes.

Eventually, the engineers developed methods whereby the large amount of water could be abandoned. Nozzles had to be designed for distributing very small quantities of liquid uniformly. Here the experience obtained by engineers in the combustion of liquid fuels was tapped. This led to the development of hydraulic low volume spraying. Quite another type of low volume equipment is the air-blast low volume sprayer, the so-called mistblower or atomizer. A large variety of low volume sprayers was constructed, taking into account the crops that had to be sprayed and also the method of application. For application of chemical sprays from the air, for instance, the droplet size must not be too small ( $\text{MMD}^* > 100 \mu$ ) otherwise they drift away in the wind and never reach the leaf surface. Moreover, very small droplets ( $\text{MMD} < 10 \mu$ ) will not adhere to plants even if they are applied by ground equipment.

The first blowers for low volume spraying were developed as early as 1934 (French, 1934). At that time it was thought necessary to use oil as a carrier instead of water because one feared that water would evaporate too rapidly. Later it became evident that this is not the case unless the climate is too dry.

It is interesting to note that the development of low volume spraying for agricultural purposes followed different ways in the various countries. In the United States, England, and Holland for instance, the cold method was mainly used, whereby the liquid was atomized into tiny particles by means of a very fast centrifugal fan or by using compressed air. In Germany on the other hand, attention of the research workers has long been concentrated on aerosols, i.e., a dispersion of liquid with a mass median diameter (MMD) of less than  $40 \mu$ . They started with the so-called hot method, already known in the United States, whereby superheated steam or exhaust gases were used as atomizers and propellents for material dispersal. In the latter case particles are so small that they are carried a long distance by even low wind velocities. Therefore, up to now this method has mainly been successfully applied in woods (e.g., for the control of cockchafers) or in barns. In the laboratory, it has also been possible to use fungicides in this way but no practical field controls have been obtained because spray drift takes the fungicidal clouds over too large a distance, and it does not settle well. In Germany, Stobwasser (1953) especially investigated the possibilities of this method.

Engineers have been studying the behavior of the liquid sheet pro-

\* MMD = mass median diameter.



duced by different designs of nozzles but up to now there is no connecting theory to enable them to predict the performance and to guide them in their design (Fraser, 1957). Nevertheless, the postwar development of low volume machines has been amazing (Ripper, 1955; van den Muijzenberg, 1957).

A new and promising engineering development is electrodusting, whereby the dust is given a charge (Bowen *et al.*, 1952). Thus, two to three times as much dust settles on the leaves as compared with non-charged dusts. There is little or no clogging of dust particles and deposits are much more uniform; moreover, there is a good deposit at the underside of the leaves. Tenacity is much better against wind and mechanical movements but rain resistance is only slightly better than with uncharged dusts (Göhlich, 1957). This type of dusting looks very attractive for areas with a poor water supply.

Interesting machines have been built for the application of chemicals to the soil, e.g., for the control of nematodes.

#### 11. *Physics, Providing Essential Tools and Methods of Control*

At the very dawn of plant pathology as a science the great importance of physics for the study of plant diseases was realized. In the first handbook on plant pathology, Kühn (1858) dedicates more than 30 pages to the description and use of the compound microscope, an invaluable tool to the plant pathologist. Without this instrument it would have been impossible to distinguish the morphological characters of the causal agents of parasitic diseases and to study their life cycles. By using the polarizing microscope, Takahashi and Rawlins (1933) got strong evidence of the shape of tobacco mosaic virus before it could be observed with the electron microscope.

Another optical tool is the ultraviolet spectrophotometer. Ultraviolet absorption spectra contribute to the study of nucleoproteins (Fraenkel-Conrat and Williams, 1955) and make possible the determination of concentrations of highly purified virus preparations (Takahashi, 1951). However, this method is not very specific. Ultraviolet fluorescence is used for diagnostic purposes, e.g., in the case of boron deficiency in celery called brown checking. While normal young growing petioles appear a dull reddish green, early brown checking symptoms on the inside of the petiole exhibit a bright light blue fluorescence even at a time when no symptoms are visible on the outside (Spurr, 1952). A disadvantage of the use of blue fluorescence may be that with other crops the same change of color is obtained if necrosis is due to different causes. Tubers of potatoes having internal necrosis initiated by the dry rot fungus *Fusarium caeruleum* display a blue fluorescence under ultra-



violet light, but the same color can be seen if they are attacked by *Corynebacterium sepedonicum*, the ring rot bacterium. Moreover sprain, probably a virus disease, gives about the same fluorescence. Yellow fluorescence, as also described by Spurr for brown checking of celery, may be more specific. The type of color of the fluorescence enabled chemists to identify some of the causal agents. Frequently, coumarins have been found. They may play a role in the development of necrosis. The sterilizing effect of ultraviolet light, especially on bacteria, has led to the frequent use of ultraviolet tubes in sterile transfer rooms.

The other side of the spectrum, the infrared, has also been used in plant pathology, e.g., for the disinfection of chestnuts imported into France from countries where the dangerous chestnut blight (*Endothia parasitica*) occurs (Busnel *et al.*, 1951). The advantage of using heat treatment obtained by infrared irradiation over heating by convection in dry air is obvious in this case: 80 seconds in an apparatus heated with infrared radiation (12,000 Å), killed all spores of *Endothia*, whereas a normal heat treatment of 10 minutes at 100° C. gave only a very incomplete sterilization of the fruit surface.

X-ray radiation has been used in virus research, e.g., for investigations of the structure of some viruses; it can also produce new mutants of either plants or fungi.

Several of the latest developments in physics are being investigated for their possibilities for research in the field of plant pathology or for the control of plant disease, e.g., the effect of ultrasonic waves and more recently of atomic energy.

Ultrasonic treatment using special equipment has been successful for the control of plant pathogenic bacteria (*Bacterium michiganense* in tomato seed) and fungi present under the seed coat (*Phoma betae*, *Cercospora beticola*, etc., in beet seed), if the treatment was combined with special fungicides (e.g., 8-oxyquinoline sulfate) which alone proved ineffective (Jaenichen and Heimann, 1955). High intensity ultrasonic waves changed the physical structure and infectivity of tobacco mosaic virus (Newton, 1951).

Another interesting use of ultrasonic energy was recently described by Waid and Woodman (1957). It appears that the transmission of ultrasonic waves through wood is considerably reduced in case of even very slight inner decay. Fungal infections such as heart rot often remain invisible on the outside of a tree; so they may be destructive for years. Through this new technique, inner diseases of trees and wood can be detected in an early stage and measures can be taken, leading to a marked reduction in economic loss of timber.

Use of beams of electrons was made by plant pathologists, more

particularly virologists, when they started using the electron microscope in their studies. Magnifications of 10,000 to 30,000 times (now even up to several hundred thousand times) made virus particles visible for the first time; the instrument is now indispensable for diagnostic purposes in virus research and for studies on the behavior of virus particles inside the cell.

The latest and most fascinating developments in physics are concerned with the structure of atoms and the possibilities of mastering the immense forces being liberated by atomic fission. From nuclear reactors, radioactive isotopes are readily available and are of special interest for research in medicine, biology, and agriculture. They can be incorporated into chemical compounds such as fungicides and these so-called labeled fungicides can easily be followed on their way in plants and fungus tissues, in extremely minute amounts. It appears that all fungicides tested, with the exception of sulfur, are accumulated by fungus spores (Miller and McCallan, 1956). Radioactive fungicides and nutrients have shown a marked accumulation at infected sites of leaves, especially if obligate parasites were used in the experiments (Miller, 1956).

In plant pathology and plant breeding radioisotopes are useful as a source of ionizing radiation. As a source of gamma rays,  $\text{Co}^{60}$  is convenient for the induction of inherited changes in germ plasm and for sterilizing biological tissues (especially in food preservation). With this mutagenic agent the plant breeder can produce resistant varieties of valuable crops more efficiently than with the usual plant breeding methods (Myers *et al.*, 1956). Also, one can produce more virulent races of the pathogen. Under controlled conditions, the breeder could use such races to breed adequate resistance prior to the appearance of new strains of the pathogen in the field (Anonymous, 1956).

Electrophoresis and the ultracentrifuge are complementary physical tools essential for the study of the basic properties of size, shape, and electrical charge of viruses.

These few examples clearly demonstrate the important contribution physics has made to a better understanding of the causes and backgrounds of plant diseases and the ways by which they may be controlled.

## 12. Meteorology Necessary for Epidemiology, Ecology, and Phenology

There is hardly any parasitic disease that is not influenced by the climate, and many diseases derive directly from adverse weather conditions. In warm continental regions crop failures due to drought or heat are common, whereas in the temperate zone late frost may do a lot of damage. Thus, meteorology is another science that is intimately related

to plant pathology. These relations will be developed further in Chapter 14 of this volume and in various chapters of Volume III.

Since L. R. Jones and his colleagues in Wisconsin carried out their classic experiments on the influence of soil temperature on cabbage yellows, plant pathologists have paid considerable attention to the influence of environmental conditions on the development of plant diseases. Literature on this subject comprises about one-tenth of all phytopathological papers (Foister, 1946).

As "weather" is a complicated phenomenon, most plant pathologists investigate its influence under laboratory conditions, e.g., the influence of temperature and humidity on the disease syndrome and on the pathogen, while keeping other environmental factors constant. Afterwards they translate their findings into actual field conditions. Thus, many important results have been obtained which have led to a better understanding of pathogenesis and the epidemiological spread of the diseases under investigation.

One typical example may be cited. In the Fall of 1949 turnips, a common catch crop on the sandy soils in the eastern part of The Netherlands, were severely damaged for the first time by a disease which appeared to be caused by the nonpersistent turnip virus 1. Beemster (1957) proved that severe symptoms developed only at 20–25° C. Plants grown at 15° C. did not show any reaction, but after transferring such plants to 20–25° C. necrotic symptoms appeared within 3 days. Temperature data from the Royal Netherlands Meteorological Institute showed that September 1949 had been the warmest September since daily temperature measurements were started more than 200 years ago. Because of this, work on this disease was stopped, since it was extremely improbable that the disease would again be of economic importance in the near future.

High temperature, on the other hand, may mask symptoms of other virus diseases (e.g., the effect of cauliflower mosaic virus; Walker *et al.*, 1945).

With most fungus diseases both temperature and humidity are of major importance for disease development. In the case of apple scab infection, for instance, there is a close correlation between temperature and the time that the leaves must be wet.

Only careful investigation of each separate disease can lead to the establishment of optimum temperature and humidity conditions for infection. These conditions in many cases are quite distinct from the optimum for fungus growth on cultural media.

Damage to plants as a result of industrial air pollution is also depen-

dent on meteorological conditions, the wind direction being responsible for the place where damage occurs, fog or drizzle often increasing severity of symptoms.

Hail storms, wind, and frost may cause considerable damage not only directly but also indirectly as several weak pathogens may become destructive once they have been able to enter plant tissues through mechanical lesions. Miss Kerling (1953) was able to demonstrate in laboratory trials that young pea plants treated with an air stream containing fine sand particles with or without water, were much more severely affected by *Fusarium avenaceum* than plants treated with a water stream only.



FIG. 1. Circular experiment field for the study of the spread of a disease in relation to weather conditions: Diseased plants are planted in the center. Temperature and humidity are registered by the instruments to the right.

Wind, carrying spores of fungi, is often responsible for the spread of such diseases as stem and yellow rust of cereals even over large distances, thus leading to real epidemics. Van Doorn and Post (unpublished) designed a circular experiment field—divided into 16 sectors and separated by narrow open strips (see Fig. 1)—in order to investigate the relation between spread of a disease and wind direction. Thus, a correlation could be established between wind direction during a period critical for infection and the sector in which disease outbreak was most severe. The method has been successfully used in The Netherlands for experiments with downy mildew in onions, late blight of potato, and apple mildew.



Annual records of phenological data, such as the first discharge and germination of ascospores of *Venturia inaequalis*, are of great value for disease-warning services on which the growers rely with their spraying scheme.

The best and most reliable analysis is obtained if plant pathologist and meteorologist work closely together. The development of epidemics and the possibilities of forecasting them will be treated in Chapter 8 of Volume III.

### 13. Soil Microbiology and Soil Chemistry and Their Importance to Soil-Borne Plant Diseases

The study and the control of soil-borne plant diseases is one of the most difficult subjects in plant pathology, for one reason because "soil" is a very complex substrate largely differing from one place to the other as to physical, chemical, and biotic properties.

With respect to physical properties, the following quotation of Sir John Russell (1957) is of interest to the plant pathologist: "It seems incredible but nevertheless it is true that in an apparently solid clod of earth only about half is usually solid matter, the other half is simply empty except for the air and water it contains." If this is the case, one can easily understand why microorganisms, nematodes, insect larvae, etc., thrive in inconceivably large numbers in most soils. These organisms are not only influenced by the chemical constitution, the pH, the texture, and the water-content of the soil, but also by such environmental conditions as soil temperature and by the presence or absence of antagonistic fungi or bacteria and, in the case of nematodes, by the number of predators (e.g., other eelworms) or parasites [e.g., amoebae (*Theratomyxa weberi*, van der Laan, 1954), nematode catching fungi such as *Arthrobotrys oligospora* and *Dactylella* spp. (Deschiens *et al.*, 1943)].

Another complication arising for the soil microbiologist is the influence of growing plant roots on microbial life in the direct vicinity of such roots. In this so-called rhizosphere, the microbial flora differs from that of soil remote from the roots, not only quantitatively (100 times more bacteria were present near the root than in the adjacent soil) but also qualitatively (Garrett, 1955a).

Root exudates are responsible for the hatching of the cysts of highly specific parasitic nematodes such as *Heterodera rostochiensis* and *H. schachtii* and for the positive chemotaxis found with several other eelworms. In some cases the root secretions are nematocidal, e.g., with African marigold (*Tagetes erecta*). The chemical responsible for this nematocidal action has been isolated and was identified as alpha ter-



thienyl (Uhlenbroek and Bijloo, 1957). Root secretions of tulips and other plants have been found to be fungitoxic (Winter and Willeke, 1951). It is highly probable that these secretions prevent parasitic fungi from passing through the rhizosphere.

Not only do parasitic nematodes, fungi, and bacteria stay over in the soil, either in a resting stage or as mycelium, but during the past few decennia it has been found that several viruses also remain virulent in the soil during the dormant season when no crop plants are present. How these viruses (nucleoproteins) remain unchanged in such an active microbiological and chemical medium is not quite clear. They may be adsorbed to clay minerals like montmorillonite (van der Want, 1951) or they may overwinter in plant material either derived from the previous year's crop or in the roots of surviving weeds (Noordam, 1956). It has also been suggested that they bridge unfavorable periods inside some kind of vector (McKinney *et al.*, 1957). Recent unpublished investigations support this hypothesis. Eelworms as well as fungi have been found to act as vectors for such viruses.

It is a well-known phenomenon that in general disease symptoms are more severe and that the pathogen develops more rapidly and intensely in sterilized soil than in normal untreated soil. This led Garrett (1955b) to the conclusion that the normal microflora of the soil exert a natural biological control of most, if not all, soil-borne diseases of plants. In several cases, the parasitism of certain soil-inhabiting fungi or bacteria toward plant pathogens has been proved experimentally, e.g., with *Rhizoctonia solani* being destroyed by *Trichoderma viride* (Weindling, 1932). From this fungus the antibiotics gliotoxin and viridin could be isolated. *Penicillium expansum* and *Pullularia pullulans* exerted a strong antagonistic effect against *Pythium volutum* and *P. debaryanum*, causing root decay of grasses (van Luyk, 1938).

These complex interactions among soil organisms are discussed more fully in Chapter 8 of Volume II.

Soil chemistry is mentioned in the heading of this paragraph because lack or unavailability of certain elements in many cases induces nutritional diseases in plants. Even lack of so-called trace elements can be, and in fact often is, very harmful to our crops. Because soil is a very complex substrate, the mere presence of an element does not at all guarantee its availability to plants. Iron deficiency for instance may be the result of toxic amounts of heavy metals or an excess of calcium carbonate. It produces typical chlorotic symptoms. The application of high amounts of potassium fertilizers may block the availability of magnesium, symptoms showing especially in plants with a low nitrogen content (Russell, 1957). Not only does the amount of available elements

essential for plant nutrition determine whether nutritional diseases will develop, but also the ratio of these elements is of great importance for the predisposition of crop plants towards parasitic diseases.

From the soil scientist the plant pathologist derives his ideas for the solution of all these diverse problems.

#### 14. *Conclusions*

From the preceding paragraphs it will be clear that our knowledge of plant pathology and the control of plant diseases has grown greatly since the foundation of this science a century ago. Our basic insight into what really happens in a diseased plant remains still very limited, but we now know for certain that biochemical processes are involved which seem to be responsible for pathological physiology and pathological morphology.

Biochemistry, plant physiology, and plant morphology have all profited from the work of plant pathologists. Tobacco mosaic virus, being responsible for typical disease symptoms, is today one of the favorites of the biochemist from which he obtained considerable information on the structure of proteins. Moreover, these studies have contributed to our knowledge of nucleic acids, most essential substances in the protein synthesis in general.

Plant pathologists were the first to be interested in the study of chemical activities of fungi, a rather new field of research of major importance for fundamental biochemistry (Foster, 1949) and for the development of antibiotics to which mankind owes so much.

Basic studies on the translocation of virus in sugar beets (Bennett, 1937) led to a better understanding of translocation of food in plants.

Some virus diseases causing phyllody and proliferations have furnished valuable facts for the solution of morphological problems, e.g., for the interpretation of floral morphology. They supported Goethe's theory that the flower must be regarded as a modified leafy branch (Bos, 1957).

The plant pathologist knows how to make use of genetics, plant taxonomy, and plant geography for the production of resistant varieties. Breeding for disease resistance in its turn has had a stimulating effect, not only on many breeding programs, but also on the accumulation of basic knowledge of the genetics both of higher plants and of fungi. The study of mycology and virology led to a better understanding of pathogenesis and enabled us to employ chemical and physical control measures which otherwise would have been impossible. Plant pathologists have described many new fungus species and their life cycles; they also contributed to fungus physiology, genetics, and biochemistry. Chemists and technologists furnished many powerful weapons in man's

fight against such enemies as insects, nematodes, bacteria, and fungi. But did not the plant pathologist, the applied entomologist, the nematologist, and the bacteriologist by their discoveries initiate this whole new field of chemical activities resulting in huge chemical industries with an annual turnover of several hundred million dollars?

Modern, most ingenious physical instruments stand at the disposal of the plant pathologist today. On the one hand this provides him with better possibilities than his ancestors, but on the other hand it also increases his responsibilities: he has less excuse for not finding control measures for serious plant diseases. In the meantime it may be wise to remember that it is hard to change weather conditions which have such a strong influence on pathogenesis and epidemiology.

The practical plant pathologist should take great care not to overlook the experience gained by countless generations of farmers in the course of many centuries. Prevention is better than cure and good crop husbandry (including plant hygiene) will forever remain a cheap and promising basis for the growing of healthy crops. But it is by no means always successful nor are control measures known against all diseases. This leaves us with some serious problems. However, we may hope to solve many of them in the future if we are prepared to cooperate with specialists from the most divergent disciplines. This seems an absolute necessity because of the growing interrelationships between all natural sciences.

As the world population increases rapidly and as already an alarming percentage of our contemporaries are underfed or starving it is our duty to intensify our efforts to increase crop yields, amongst others by controlling plant diseases. Only if scientists from all over the world have a free exchange of their results and ideas may we ever hope to reach our goal: sufficient food for every human being living on the surface of the earth.

It is our intention to give an impression of some aspects of the fight against plant diseases, their influence on human society and history in the next pages.

### III. PLANT PATHOLOGY AND HUMAN SOCIETY

Outbreaks of plant diseases have greatly influenced human society since the beginning of the cultivation of crop plants by man. Sometimes they have resulted in famines. Even today, losses may be tremendous. In the United States alone, losses due to plant diseases are estimated at about  $3 \times 10^9$  dollars a year (Wood, 1953); i.e., the equivalent of 7% of the total potential production.

Already about two-thirds of the world's population is underfed,

hungry, or starving, and it is impossible to enlarge the area of cultivated land sufficiently to provide enough food for the existing millions. And still, the world population is increasing by about 30,000,000 people a year, or the equivalent of a new Australia to feed every 4 months (Stakman, 1957). Therefore we must raise the production per acre and here the plant pathologist has an important task to fulfill.

#### A. Influence of Plant Diseases on the Production of Food and Raw Materials

Plant diseases have wiped out or seriously threatened many flourishing tropical plantations. Coffee rust caused by *Hemileia vastatrix*, toward the end of last century, destroyed the culture of Arabian coffee on Ceylon (see also Section III, B, 4) and *Dothidella ulei*, causing South American leaf blight of rubber (*Hevea brasiliensis*), made the cultivation of this native tree impossible until an ingenious multiple grafting technique was adopted (private communication by Dr. Lee Ling, F.A.O.).

In order to establish a soft wood industry the Forest Department of Kenya started plantations of cypress (*Cupressus macrocarpa*). Growth was abundant until the trees were about 20 years old. Then, *Monochaetia unicornis*, causing severe cankers of the trunk, spread rapidly either killing the trees or making the timber worthless (Watts Padwick, 1956).

Tobacco plants on Sumatra, producing the world famous cigar wrappers, suffered frequently from an unknown top necrosis, until Mes (1930) revealed that this disease was a result of boron deficiency.

Leaf spot or Sigatoka disease of bananas caused by *Cercospora musae* has become so serious in Central America that without the use of fungicides (15–17 sprayings per year) the banana industry could not survive in that area.

Breeding of resistant varieties has saved a tropical crop in other cases. One of the oldest examples is the breeding of sugar cane varieties resistant against sereh-disease and mosaic, both caused by viruses (see also Section II, B, 3). Sugar beet varieties resistant to curly top (also a virus disease) saved the sugar beet crop for the western United States. Following the introduction of these resistant varieties the sugar beet area in California increased from 53,000 to 170,000 acres and the yield from 8.3 to 16 tons per acre, whereas before, several sugar factories had to be closed down as a result of the disease.

Bacterial blight or black-arm, a cotton disease caused by *Xanthomonas malvacearum*, has been particularly destructive in several cotton producing countries in Africa. In Uganda the introduction of seed dressing with a copper fungicide combined with the use of a more resistant



cotton variety resulted in a doubling of the yield if compared with the previous 27 years (Pottie, 1953).

The risks taken by the farmer in producing a crop and how he can combat them are familiar to all plant pathologists. The ratio of benefit received to cost of a control measure determines how far the farmer can go in applying control measures. Although the diseases are different, the food merchandising organization is confronted with the same problem, because their commodity is perishable and marketing diseases take their toll of produce in transit and storage.

## B. Social Effects of Plant Diseases

### 1. Reduction of World Food Supplies

Since, as we have already seen, two-thirds of mankind have not enough to eat, it is a deplorable fact that weeds, diseases, and pests take about 20% of the food production of the world. This is a low estimate since it is based on figures provided for the United States, a country with high agricultural standards and extensive crop protection. From this 20% loss, 7% is due to plant diseases.

Every plant pathologist who has been working in the tropics, will agree that crop losses are much higher there, partly as a result of primitive farming and inadequate disease control, partly because a humid hot climate favors epidemics of many fungus diseases. Moreover, losses during storage are much higher in less developed countries.

Although Asia produces about as much food as Europe and the United States together, the people of many Asiatic countries are very vulnerable with respect to their food situation. A sudden outbreak of a serious disease in an essential food crop may soon cause a famine, for food reserves are very limited or nonexistent.

White stripe (*hoja blanca*) is a destructive virus disease of rice, resembling rice stripe, known for a long time from Japan. Since about 1954, this disease has been found in the Western Hemisphere where it caused a reduction of rice yields of 25 to 50% in countries like Cuba and Venezuela. Recently it has been reported also from Panama (Lasaga, 1957) and Florida.

Another serious disease of an essential food crop is maize rust (*Puccinia polysora*) which fungus disease has spread very rapidly over large parts of the world since the last World War. It is especially serious in West Africa where loss in corn grain amounted to 40% in 1951 (Watts Padwick, 1956). It is a tropical rust and does not occur under cool conditions.

The devastating effect of rust epidemics in wheat is well known.



As wheat, rice, and maize produce the bulk of the world's food one can imagine what damage is done by such diseases as have just been mentioned.

There is not much chance for a sufficient expansion of the agricultural area of the earth. To a small extent this may be done by irrigation of desert areas (Middle East, United States, North Africa), reclamation of inland seas (Holland) or deforestation, but this never will suffice to feed the rapidly increasing population of the world. The only alternative is a higher yield per acre. This may be reached by using better producing varieties (hybrid corn), virus free seed stocks (potatoes), fertilizers, disease resistant crops, and disease control through plant hygiene and the application of chemicals, such as fungicides, herbicides, insecticides, and nematocides.

## 2. Local Famines in Past and Present

On the basis of information in the scarce literature of ancient times it seems highly probable that famines as a result of rust and mildew epidemics on wheat were not uncommon in the Roman empire.

The serious famine resulting from the failure of the potato crop in Ireland in two successive years (1845 and 1846) is so well known, that we hardly need mention it here. Some consequences will be dealt with in Section III, C of this chapter. The fungus that caused this disaster, *Phytophthora infestans*, is still responsible for heavy losses. In some years it may destroy over 10% of the world's potato crop. As the annual world production of white potatoes is estimated at 8 billion bushels, this means a loss of no less than 800,000,000 bushels or about 22,500,000 tons of valuable food.

However, not the potato, but cereal crops are the most important ones for human subsistence throughout the world, providing roughly 80% of man's food. Among the cereals, the production of wheat ranks highest. It has been estimated that for the world as a whole the annual average losses caused by rust fungi amount to about 600,000,000 bushels. In the center of a stem rust epidemic susceptible varieties may even have a yield reduction of 80%. If no food is supplied from elsewhere, this may easily initiate a famine.

A rice crop failure in Bengal due to *Helminthosporium oryzae* has actually led to famine conditions.

Cassava, although not nearly as valuable a food as rice, nevertheless is an important subsistence crop in the tropics. Mosaic, a virus disease, frequently occurring in African cassava, may cause a severe reduction in yield. Cassava is a vegetatively propagated crop and, e.g., in Uganda it was totally infected with mosaic, yielding 2 tons an acre, whereas

selected virus free stocks yielded 14 tons to the acre. This means that in the Uganda district where the virus free stock was introduced, each man, woman, and child now gets one extra pound of food a day (Int. Conf. Crop Protection, Fernhurst, 1951).

The sudden outbreak of maize rust has in some areas (West Africa) reduced the yield by 40%, thus endangering the local food position.

### 3. Poisoning of Food Due to Plant Diseases

Poisoning of man and livestock animals as a result of plant diseases does not occur frequently today. In the Middle Ages, however, due to eating ergot-poisoned rye bread thousands among the poorer classes of Europe have suffered terribly from ergotism or St. Anthony's fire as it was often called. In severe cases of gangrenous ergotism the patients lose fingers, toes, or even whole limbs, the flesh gradually rotting away. Another type called convulsive ergotism, is characterized by nervous symptoms which in severe cases led to convulsions of the whole body and finally various mental derangements such as epilepsy and dementia (Barger, 1931).

Not before the first half of the 17th century was the cause of this disease discovered; that is to say it was then that a connection was found between the occurrence of many large kernels (so-called ergots) in rye fields and the outbreak of ergotism after eating bread, made from ergot-contaminated flour. But it was not until the middle of the 19th century that the cause of the ergot-formation was identified and the life cycle of this parasitic fungus (*Claviceps purpurea*) was unraveled by the well-known French mycologist Tulasne. He also proved that the ergot was the sclerotium of this fungus and not an abnormal outgrowth of the ovary of the rye kernel.

Less severe poisoning of man may result from eating bread made from rye heavily infected with *Fusarium* spp. or *Phialea temulenta*. Symptoms are general weakness, vertigo, nausea, and headache.

*Claviceps purpurea* is not restricted to rye; it may infect at least 150 different grasses and thus cause ergotism in cattle with symptoms similar to those described for man. It frequently leads to abortion (Hardison, 1953).

Barley seed infected with *Gibberella zeae*, is often found in the eastern and central United States. It is called "scabby" grain and, when fed to pigs, it appears to be poisonous.

Crown rust (*Puccinia coronata* var. *lolii*) renders perennial rye grass (*Lolium perenne*) unpalatable to sheep in New Zealand (Cruickshank, 1957).

The nematode *Anguina agrostis* is a common parasite of grass seed,

particularly of chewings fescue (*Festuca* sp.). Fatal poisoning of sheep, cattle, and pigs has occurred in western Oregon from feeding screenings of this grass seed containing nematode galls (Hardison, 1953).

Also mineral deficiencies in forage crops and grasses may lead to abnormalities or diseases in animals. Seeds from manganese deficient oats fed to poultry induced cannibalism, perosis, and bad hatching of eggs. Copper deficiency of meadow grasses is manifested by unthriftiness, depraved appetite, anemia, and diarrhea in cattle. Applying copper sulfate to the deficient meadow or directly to the animal causes disease symptoms to disappear.

#### 4. *Why the English Drink More Tea Than Coffee*

In England consumption of coffee was about equal to that of tea in the middle of the 19th century (Ordish, 1952). At that time, Ceylon was one of the world's greatest coffee producing countries, followed immediately by India, Malaya, and Java.

In 1867, a pathogenic leaf fungus, later known as coffee rust (*Hemileia vastatrix*), was found in one coffee plantation of Ceylon. This pathogen was spread with alarming rapidity by the monsoon winds, favored by the uninterrupted monoculture of coffee over large areas.

While in 1871 the average annual yield was still about 4.5 cwt. (= 228.6 kg.)/acre it had dropped in 1878 to 2 cwt. (= 101.6 kg.)/acre. This, for Ceylon alone, meant a monetary loss of about \$5,000,000. Between 1879 and 1893 exports of coffee dropped to less than 7% of the pre-rust epidemic shipments (see Wellman, 1953). No wonder, then, that *Hemileia* ended coffee growing on a large scale, not only in Ceylon, but also in other East Asiatic countries. "The planters (on Ceylon) were ruined and the Oriental Bank went smash in the general confusion" (Large, 1946).

After this catastrophe Brazil gradually became the main coffee producer of the world. Fortunately for that country, coffee rust is not known in the Western Hemisphere, but with modern rapid transportation there is always the very real danger of importation, just as maize rust is now spreading with alarming speed all over the world.

Coffee rust is also present in British East Africa, but there it produces serious losses only occasionally. By spraying with copper and other fungicides, leaf fall can be reduced by 10 to 40% (Watts Padwick, 1956).

After shifting from coffee to tea, the Ceylon plantations gradually became profitable once more, until blister blight threatened them again. However, very effective chemical control measures have been developed against this disease.

Since the coffee rust disaster, tea has become much more important

in Great Britain and Ireland. While coffee and tea consumption were about equal in the middle of the last century, there is now a 6 : 1 ratio in favor of tea (Ordish, 1952). That the British did not import coffee from Brazil on a large scale, but changed their drinking habits, is probably due to the favorable trade relations existing within the British Empire and, in more recent times, between the partners of the Commonwealth.

#### 5. *Development of Chemical Industries, Dusting and Spraying Equipment Firms, and Spraying Contractors*

Especially during and after the Second World War the number of potent crop pesticide products increased tremendously. It is estimated that the annual world sale of these products amounts to approximately \$280,000,000 at wholesale prices, or an on-farm value of \$420,000,000 (Ordish, 1952). How much of this money is spent for fungicides is not known. But also, without this knowledge it will be clear that many people find a living in the production, distribution, and sale of such chemicals.

In Section II, B, 10 of this chapter a short survey was given of the newer types of machines developed for the application of pesticides. Like the chemical plants, the machine manufacturers employ many people.

For the designing of new sprayers, blowers, and dusters and for the search for new fungicides, insecticides, etc., industrial firms have invested large sums in laboratories, their staff, and equipment.

Spraying, especially with the more poisonous chemicals, must be done with great precaution and skill and can best be carried out by specialists. As a result more and more acres are treated by contract sprayers.

A special kind of contract spraying is carried out by aerial spraying companies, well known for instance from locust campaigns in the Middle East and elsewhere. This type of spraying is suitable only for larger areas and in most countries where it is employed its use is limited to insect control, weed control, or the aerial application of fertilizers. Recently it became clear that fungicides could also be distributed successfully this way, e.g., on potato fields for the prevention of late blight.

#### 6. *Potential Dangers in the Use of Poisonous Chemicals on Food Crops*

"Many of us look with growing concern at the chemical warfare which farmers and horticulturists are forced to wage today against insects and diseases if they want to secure their share in the markets and make a success of their chosen profession" declared F. T. Wahlen, of the Food and Agriculture Organization, in an address at the opening session of the 7th International Botanical Congress (1950).



It is in a way gratifying for the plant pathologist that the fungicides he prescribes are, generally speaking, far less poisonous to man and livestock animals than many modern insecticides and weed killers.

But some fungicides, such as organic mercury compounds, can also be dangerous for public health when used as foliage sprays. They remain on the leaves and young fruits for weeks, are easily absorbed by the waxy layers of the fruit skin and may persist there until after harvest. Because they are cumulative poisons, Public Health Authorities in most countries do not allow even traces of mercury residues on fruits and vegetables. This practically will prohibit their use as foliage sprays, e.g., against apple scab, where they have certain advantages as eradicants.

Whether or not a new pesticide will be accepted for general use, therefore, depends not only on its effect against the noxious organism, but also on its toxicity for man and livestock animals. This is investigated in many countries by Public Health Institutes and approval or disapproval is finally decided by such organizations as the Food and Drug Administration (United States) or by special Committees in which both Public Health Administrators and Officers of Plant Protection Organizations participate (England and Western European countries).

Danger for the consumer can be reduced to acceptable limits by either prohibiting the use of certain chemicals or by restricting their use to certain periods. Although there have been some incidental cases of poisoning of consumers none is known to have been fatal.

The man who applies the chemicals to the crop runs more risks, but if he is careful and keeps to the instructions given he need not be worried too much.

Adverse effects on livestock animals have been observed, e.g., if spray drift brought arsenic weed killers on adjacent meadows.

A danger of quite another type is poisoning of the soil by too frequent uses of pest control chemicals. Up to now this has been observed in orchards in western United States where manifold applications of arsenic insecticides made replanting of apple trees impossible. Arsenic compounds used as weed killers may poison arable land. Oats sown after such treatments of a previous crop have shown a marked reduction in growth and yield.

Too frequent spraying of copper fungicides on the foliage of crops has also resulted in unfavorable effects on plant growth.

### *C. Plant Pathology and Human History*

Plant diseases never had such a direct bearing on human history as those epidemic diseases of man in which insects played a major role. "Applied entomology and human history" therefore would yield more impressive facts.



What happened in ancient times we do not know. It is quite possible that famines resulting, for instance, from losses of wheat by rusts have had influence of historic importance.

In more recent times the most striking example of how a plant disease may influence history is the case of the great famine in Ireland toward the middle of the last century. In 1845 and 1846 late blight almost wiped out the whole potato crop in that country. This together with the very backward social and political situation not only led to the Irish famine and the emigration of hundreds of thousands of Irishmen to the United States, but also it was a decisive factor in the subsequent social and economic policy in Ireland itself. It ultimately led to the separation of Ireland from the United Kingdom.

The Irish famine has greatly stimulated research in the field of plant diseases and may be seen as the start of the modern era of plant pathology, which since has yielded so many results of great economic and social importance.

Epidemics of plant diseases in the tropics have often had political repercussions, especially if the threatened crop is the one of greatest economic importance to the community. A typical example is the swollen shoot disease of cacao, reducing the production in the eastern region of the Gold Coast from 128,000 tons in 1936-37 to 47,583 tons in 1951-52. In some areas production was reduced by as much as 80%. In 1953 there were about 50,000,000 infected trees. By cutting out the diseased trees, the rural industry of the Eastern Province of the Gold Coast was upset, and the expensive cutting out program has had serious political repercussions (Watts Padwick, 1956).

Good control of plant diseases is essential for the maintenance of a better standard of living, especially in the underdeveloped countries.

Thus, by helping to increase the world's food supplies, plant pathologists may have some influence on human history in underdeveloped regions. It must be admitted, however, that this influence will only be very small as long as the people of such regions do not wholeheartedly cooperate in our fight against plant diseases and insect pests.

#### ACKNOWLEDGMENTS

The author is very grateful to several specialists from various scientific institutes, including his own, for critical reading of the first 14 paragraphs. He feels indebted to Miss J. M. Krythe, M.Sc., documentalist of the Institute for Phytopathological Research at Wageningen for her help in selecting the literature and for preparing the list of references. The author is much obliged to Dr. A. H. Gold, Department of Plant Pathology, University of California, Berkeley, and at the time guest-worker at the Institute for Phytopathological Research, Wageningen, who corrected the English text and who gave some valuable suggestions concerning virus diseases.

## REFERENCES

- Allen, P. J. 1942. Changes in the metabolism of wheat leaves induced by infection with powdery mildew. *Australian J. Botany* **29**: 425-435.
- Andrus, C. F., and B. L. Wade. 1942. The factorial interpretation of anthracnose resistance in beans. *U. S. Dept. Agr. Tech. Bull.* **810**: 1-29.
- Anonymous. 1956. The uses of atomic energy in food and agriculture. *Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1955* **12**: 10-18.
- Bald, J. G., and G. Samuel. 1931. Investigations on "spotted wilt" of tomatoes. II. *Australian Commonwealth Council Sci. Ind. Research Bull.* **54**: 1-24.
- Barger, G. 1931. "Ergot and Ergotism." A monograph. Gurney & Jackson, London. 279 pp.
- Bawden, F. C., and N. W. Pirie. 1937. The relationships between liquid crystalline preparations of cucumber viruses 3 and 4 and strains of tobacco mosaic virus. *Brit. J. Exptl. Pathol.* **18**: 275-291.
- Bawden, F. C., and N. W. Pirie. 1938. Crystalline preparations of tomato bushy stunt virus. *Brit. J. Exptl. Pathol.* **19**: 251-263.
- Beadle, G. W. 1945. Biochemical genetics. *Chem. Revs.* **37**: 15-96.
- Beemster, A. B. R. 1957. Onderzoekingen over een virusziekte bij stoppelknollen (*Brassica rapa* var. *rapifera*) (Investigations on a virus disease of turnip). *Tijdschr. Plantenziekten* **63**: 1-12.
- Bennett, C. W. 1937. Correlation between movement of the curly top virus and translocation of food in tobacco and sugar beet. *J. Agr. Research* **54**: 479-502.
- Biffen, R. H. 1907. Studies in the inheritance of disease resistance. *J. Agr. Sci.* **2**: 109-128.
- Black, F. L., and C. A. Knight. 1953. Comparison of some mutants of tobacco mosaic virus. *J. Biol. Chem.* **202**: 51-57.
- Black, L. M. 1950. A plant virus, that multiplies in its insect-vector. *Nature* **166**: 852-853.
- Black, L. M., and M. K. Brakke. 1952. Multiplication of wound-tumor virus in an insect vector. *Phytopathology* **42**: 269-273.
- Black, W., C. Mastenbroek, W. R. Mills, and L. C. Peterson. 1953. A proposal for a international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. *Euphytica* **2**: 173-179.
- Bos, L. 1957. Plant teratology and plant pathology. *Tijdschr. Plantenziekten* **63**: 222-231.
- Bowen, H. D., P. Hebblethwaite, and W. M. Carleton. 1952. Application of electric charging to the deposition of insecticides and fungicides on plant surfaces. *Agr. Eng.* **33**: 347-350.
- Braun, A. C. 1955. A study on the mode of action of the wildfire toxin. *Phytopathology* **45**: 659-664.
- Brown, W. 1951. Mycology over a century. *Advance. of Sci.* **8**(30): 214-225.
- Busnel, R. G., H. Darpoux, and M. Ridé. 1951. Utilisation de la chaleur transmise par le rayonnement infrarouge comme méthode de désinfection des chataignes contre les spores d'*Endothia parasitica* (Murril) Anderson. *Compt. rend. acad. agr. France* **37**: 513-515.
- Buxton, E. W. 1956. Heterokaryosis and parasexual recombination in pathogenic strains of *Fusarium oxysporum*. *J. Gen. Microbiol.* **15**: 133-139.
- Catcheside, D. G. 1951. "The Genetics of Micro-organisms." Pitman and Sons, London, 223 pp.

- Coons, G. H. 1953. Breeding for resistance to disease. Plant diseases. *U. S. Dept. Agr. Yearbook* **1953**: 174-192.
- Cruickshank, I. A. M. 1957. Crown rust of ryegrass. *New Zealand J. Sci. Technol.* **A38**: 539-543.
- De Bary, A. 1853. "Untersuchungen über die Brandpilze und die durch sie verursachten Krankheiten der Pflanzen mit Rücksicht auf das Getreide und andere Nutzpflanzen." Müller, Berlin. 144 pp.
- De Bary, A. 1866. Morphologie und Physiologie der Pilze, Flechten und Myxomyceten. In "Handbuch der physiologischen Botanik," (W. Hofmeister, ed.), Band II.
- de Fluiter, H. J., and F. A. van der Meer. 1953. Rubus stunt, a leaf-hopper-borne virus disease. *Tijdschr. Plantenziekten* **59**: 195-197.
- Dekker, J. 1957. Inwendige ontsmetting van door *Ascochyta pisi* aangetaste erwtezaaden met de antibiotica rimocidine en pimarinine, benevens enkele aspecten van the parasitisme van deze schimmel. Ph.D. Thesis, Wageningen.
- Deschiens, R., L. Lamy, and E. Vautrin. 1943. Essais pratiques de prophylaxie de L'Anguillulose des végétaux par l'emploi d'Hyphomycètes prédateurs. *Compt. rend. Acad. Sci. Paris* **216**: 539-541.
- Dimond, A. E. 1955. Pathogenesis in the wilt diseases. *Ann. Rev. Plant Physiol.* **6**: 329-350.
- Dimond, A. E., and J. G. Horsfall. 1955. Fifty years of fungicides. *Ann. Appl. Biol.* **42**: 282-287.
- Dunegan, J. C., and S. P. Doolittle. 1953. How fungicides have been developed. Plant diseases. *U. S. Dept. Agr. Yearbook* **1953**: 115-120.
- Farkas, G. L. 1957. Biochemische Probleme der Rostkrankheiten des Weizens." Proceedings 4th International Congress of Crop Protection" (in press).
- Flor, H. H. 1954. Identification of races of flax rust by lines with single rust-conditioning genes. *U. S. Dept. Agr. Tech. Bull.* **1087**: 1-25.
- Flor, H. H. 1955. Host-parasite interaction in flax rust—its genetics and other implications. *Phytopathology* **45**: 680-685.
- Foister, C. E. 1946. The relation of weather to fungus diseases of plants. II. *Botan. Rev.* **12**: 548-591.
- Foster, J. W. 1949. "Chemical Activities of Fungi." Academic Press, New York. 648 pp.
- Fraenkel-Conrat, H., and R. C. Williams. 1955. Reconstitution of active tobacco mosaic virus from its inactive protein and maleic acid components. *Proc. Natl. Acad. Sci. U. S.* **41**: 690-698.
- Fraser, R. P. 1957. The mechanics of producing sprays of different characteristics. "Plant Protection Conference 1956." Academic Press, New York. pp. 237-277.
- French, O. C. 1934. Machinery for applying atomized oil sprays. *Agr. Eng.* **15**: 324-326, 329.
- Fries, E. M. 1821-1829. "Systema Mycologicum sistens fungorum ordines, genera, et species hucusque cognitae." Mauritius Greifswald. III Vol.
- Fuchs, W. H., and E. Kotte. 1954. Zur Kenntniss der Resistenz von *Solanum tuberosum* gegen *Phytophthora infestans* de By. *Naturwissenschaften* **41**: 169-170.
- Garrett, S. D. 1955a. Microbial ecology of the soil. *Brit. Mycol. Soc. Trans.* **38**: 1-9.
- Garrett, S. D. 1955b. A century of root-disease investigation. *Ann. Appl. Biol.* **42**: 211-219.
- Gäumann, E. 1951. Some problems of pathological wilting in plants. *Advances in Enzymol.* **11**: 401-437.

- Gäumann, E. 1954. Toxins and plant diseases. *Endeavour* **13**: 198-204.
- Gersonde, M. 1958. Über die Giftempfindlichkeit verschiedener Stämme holzzerstörender Pilze. *Holz Roh-u Werkstoff* **16**: 221-226.
- Göhlich, H. 1957. Elektrostatische Aufladung von Pflanzenschutzstäuben und -sprühschleiern." Proceedings 4th International Congress of Crop Protection Hamburg (in press).
- Grümmer, G. 1955. Die Beziehungen zwischen dem Eiweissstoffwechsel von Kulturpflanzen und ihrer Anfälligkeit gegen parasitische Pilze. *Phytopathol. Z.* **24**: 1-42.
- Hardison, J. R. 1953. Seed disorders of forage plants. Plant diseases. U. S. Dept. Agr. Yearbook **1953**: 272-276.
- Harland, S. C. 1955. Gene centres and the search for resistant breeding material. *Proc. 14th Intern. Hort. Congr.* **1**: 64-69.
- Hassebrauk, K., and R. Kaul. 1957. Vergleichende chemische Untersuchungen des Atmungsstoffwechsels von Weizenkeimpflanzen unterschiedlicher Braunrostanfälligkeit. *Phytopathol. Z.* **29**: 305-326.
- Horsfall, J. G. 1956. "Principles of Fungicidal Action." *Chronica Botanica*, Waltham, Massachusetts. 279 pp.
- Hubbeling, N. 1957. New aspects of breeding for disease resistance in beans (*Phaseolus vulgaris* L.) *Euphytica* **6**: 111-141.
- International. 1951. Control of plant virus diseases. "First International Conference Crop Protection." Plant Protection Ltd. London. Discussion. pp. 77, 81.
- Jaenichen, H., and M. Heimann. 1955. Untersuchungen über eine Anwendungsmöglichkeit des Ultraschalls in der Phytotherapie. *Phytopathol. Z.* **23**: 419-462.
- Jones, L. R. 1914. Progress in developing disease resistant cabbage. *Phytopathology* **4**: 47-48.
- Keitt, G. W., and D. M. Boone. 1954. Induction and inheritance of mutant characters in *Venturia inaequalis* in relation to its pathogenicity. *Phytopathology* **44**: 362-370.
- Keitt, G. W., M. H. Langford, and J. R. Shay. 1943. *Venturia inaequalis* (Cke) Wint., II. Genetic studies on pathogenicity and certain mutant characters. *Am. J. Botany* **30**: 491-500.
- Kerling, L. C. P. 1933. The anatomy of the "kroepoek" diseased leaf of *Nicotiana tabacum* and of *Zinnia elegans*. *Phytopathology* **23**: 175-190.
- Kerling, L. C. P. 1953. Voetziekten bij erwten, een gevolg van stuivende grond. (Footrot of peas caused by sand storms.) *Tijdschr. Plantenziekten* **59**: 62-71.
- Kühn, J. 1858. "Die Krankheiten der Kulturgewächse, ihre Ursachen und ihre Verhütung." Gustav Bosselmann, Berlin. 312 pp.
- Large, E. C. 1946. "The Advance of the Fungi," 2nd ed. J. Cape, London. 488 pp.
- Lasaga, V. 1957. White stripe—a virus disease of rice. Outbreaks and new records. *FAO Plant Protect. Bull.* **5**: 161.
- Leach, J. G. 1952. Insects, bacteria and fungi. Insects. U. S. Dept. Agr. Yearbook **1952**: 191-196.
- Lilly, V. G., and H. L. Barnett. 1951. "Physiology of the Fungi," 1st ed. McGraw-Hill, New York. 464 pp.
- Link, K. P., and J. C. Walker. 1933. The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions. *J. Biol. Chem.* **100**: 379-383.
- McKinney, H. H., W. R. Paden, and B. Koehler. 1957. Studies on chemical control



- and overseasoning of, and natural inoculation with, the soil-borne viruses of wheat and oats. *Plant Disease Repr.* **41**: 256-266.
- McNew, G. L., S. E. A. McCallan, and P. R. Miller. 1951. Treatments for vegetable seeds. *Canning Trade* **73**: 21-23.
- Markham, R. 1953. Virus nucleic acids. *Advances in Virus Research* **1**: 315-332.
- Mes, M. G. 1930. Fisiologiese siektesymptome van tabak. Ph.D. Thesis. Utrecht.
- Miller, L. P. 1956. Use of radioactive tracers in studying fungicidal action. A Conference on Radioactive Isotopes in Agriculture, Michigan State Univ. East Lansing, Michigan. pp. 223-233.
- Miller, L. P., and S. E. A. McCallan, 1956. Use of radioisotopes in tracing fungicidal action. *Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1955* **12**: 170-176.
- Müller, K. O. 1957. Über den Wirkungsmechanismus der "Abwehrnekrosen." "Proceedings 4th International Congress of Crop Protection." Hamburg (in press).
- Myers, W. M., E. R. Ausemus, F. K. S. Koo, and K. J. Hsu. 1956. Resistance to rust induced by ionizing radiations in wheat and oats. *Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1955* **12**: 60-62.
- Newton, N. 1951. Some effects of high intensity ultrasound on tobacco mosaic virus. *Science* **114**: 185-186.
- Noordam, D. 1956. Waardplanten en toetsplanten van het ratelvirus van de tabak. *Tijdschr. Plantenziekten* **62**: 219-225.
- Ordish, G. 1952. "Untaken Harvest." Constable, London. 170 pp.
- Perutz, M. F. 1958. Some recent advances in molecular biology. *Endeavour* **17**: 190-203.
- Pontecorvo, G., and G. Sermoniti. 1954. Parasexual recombination in *Penicillium chrysogenum*. *J. Gen. Microbiol.* **11**: 94-104.
- Pontecorvo, G., J. A. Roper, and E. Forbes. 1953. Genetic recombination without sexual reproduction in *Aspergillus niger*. *J. Gen. Microbiol.* **8**: 198-210.
- Pottie, J. M. 1953. Cotton growing in Uganda. *Plant Protect. Overseas Rev.* **4**: 26-29.
- Prentice, I. W. 1950. Rubus stunt: a virus disease. *J. Hort. Sci.* **26**: 35-42.
- Rhind, D., F. D. Odell, and U. T. Su. 1937. Observations on phyllody of *Sesamum* in Burma. *Indian J. Agr. Sci.* **7**: 823-840.
- Richardson, L. T. 1954. The persistence of thiram in soil and its relationship to the microbiological balance and damping-off control. *Can. J. Botany* **32**: 335-346.
- Ripper, W. E. 1955. Application methods for crop protection chemicals. *Ann. Appl. Biol.* **42**: 288-324.
- Robertson, R. N. 1957. Electrolytes in plant tissue. *Endeavour* **16**: 193-198.
- Ross, H., and M. L. Baerecke. 1950. Selection for resistance to mosaic virus (disease) in wild species and in hybrids of wild species of potatoes. The breeding of resistant varieties of potatoes. III. *Am. Potato J.* **27**: 275-284.
- Russell, E. J. 1957. "The World of the Soil." Collins, London. 237 pp.
- Saccardo, P. A. 1882-1931. "Sylloge Fungorum," Vols. I-XXV. J. W. Edwards, Ann Arbor, Michigan (1944).
- Schramm, G., and B. v. Kerékjártó. 1951. Zur Konstitution der Ribosenucleinsäure aus Hefe und Tabakmosaikvirus. *Z. Naturforsch.* **7b**: 589-594.
- Schulz, G. 1957. Vergleichende Untersuchungen mit verschiedenen Stämmen von *Lentinus lepideus*. 5. Holzschutztagung München.
- Sempio, C. 1950. Metabolic resistance to plant diseases. *Phytopathology* **40**: 799-819.
- Shaw, M., and D. J. Samborski. 1956. The physiology of host-parasite relations.



- I. The accumulation of radioactive substances at infections of facultative and obligate parasites including tobacco mosaic virus. *Can. J. Botany* **34**: 389-405.
- Shephard, H. H. 1958. Pesticide supplies and requirements. *J. Agr. Food Chem.* **6**: 188-189.
- Spurr, A. R. 1952. Fluorescence in ultraviolet light in the study of boron deficiency in celery. *Science* **116**: 421-423.
- Stakman, E. C. 1957. Progress and problems in the development of disease-resistant varieties of crop plants. "Proceedings International Congress Crop Protection." (in press).
- Stakman, E. C., M. N. Levine, and W. Q. Loegering. 1944. Identification of physiologic races of *Puccinia graminis tritici*. U. S. Dept. Agr. Bur. Entomol. Plant Quarantine E. **617**: 1-27.
- Stanley, W. M. 1935. Isolation of a crystalline protein possessing the properties of tobacco mosaic virus. *Science* [N.S.] **81**: 644-645.
- Stanley, W. M., and C. A. Knight. 1941. The chemical composition of strains of tobacco mosaic virus. *Cold Spring Harbor Symposia Quant. Biol.* **9**: 255-260.
- Stevenson, F. J., and R. V. Akeley. 1953. Control of potato diseases by disease resistance. *Phytopathology* **43**: 245-253.
- Stobwasser, H. 1953. Probleme der Anwendung von Aerosolen im Pflanzenschutz und Methodik ihrer Herstellung und Untersuchung im Laboratorium. *Z. Aerosol-Forsch. u.-Therap.* **2**: 713-729.
- Suchorukov, K. T. 1957. The physiology of immunity of some agricultural plants. "Plant Protection Conference 1956." Academic Press, New York. pp. 42-51.
- Symposium. 1953. Abnormal and pathological plant growth. *Brookhaven Symposia in Biol.* No. **6**: 303 pp.
- Takahashi, W. N. 1951. Ultraviolet absorption as a measure of tobacco mosaic virus nucleoprotein. *Phytopathology* **41**: 142-145.
- Takahashi, W. N., and T. E. Rawlins. 1933. Rod-shaped particles in tobacco mosaic virus demonstrated by stream double refraction. *Science* **77**: 26-27.
- Takami, N. 1901. Stunt disease of rice and *Nephotettix apicalis*. *J. Agr. Soc. Japan* **241**: 22-30.
- Thatcher, F. S. 1939. Osmotic and permeability relations in the nutrition of fungus parasites. *Am. J. Botany* **26**: 449-458.
- Thung, T. H. 1957. Het virologisch onderzoek aan de landbouwhogeschool, Wageningen. *Tijdschr. Plantenziekten* **63**: 209-221.
- Tolmach, L. J. 1957. Attachment and penetration of cells by viruses. *Advances in Virus Research* **4**: 63-110.
- Tulasne, L. R., and C. Tulasne. 1861-1865. "Selecta Fungorum Carpologia." Translated by W. B. Grove, A. H. R. Buller, and C. L. Shear. 1931. Oxford Univ. Press, London. (Cited by Brown, 1951.)
- Uhlenbroek, J. H., and J. D. Bijloo. 1957. Isolation and structure of a nematocidal principle occurring in *Tagetes* roots. "Proceedings 4th International Congress of Crop Protection." Hamburg (in press).
- van den Muijzenberg, E. W. B. 1957. Mistblowing and mistblowers. "Plant Protection Conference, 1956." Academic Press, New York. pp. 278-283.
- van der Kerk, G. J. M. 1956. The present state of fungicide research. *Mededel. Landbouwhogeschool Gent* **21**: 305-339.
- van der Laan, P. A. 1954. Nader onderzoek over het aaltjesvangende amoeboïede organisme *Theratomyxa weberi*. *Tijdschr. Plantenziekten* **60**: 139-145.

- van der Want, J. P. H. 1951. Some remarks on a soil-borne potato virus. *Proc. 1st Conf. Potato Virus Diseases, Wageningen-Lisse*. pp. 71-74.
- van Luyk, A. 1938. Antagonism between various microorganisms and different species of the genus *Pythium*, parasitizing upon grasses and lucerne. *Mededel. Phytopathol. Lab. Willie Commelin Scholten Baarn*. **14**: 45-82.
- van Slogteren, E. 1955. Serological diagnosis of plant virus diseases. *Ann. Appl. Biol.* **42**: 122-128.
- Vavilov, N. I. 1949-1950. The origin, variation, immunity and breeding of cultivated plants. Translated from the Russian by K. S. Chester. *Chronica Botan.* **13**: 366 pp.
- Wahlen, F. T. 1950. Plant sciences and world husbandry. *Proc. 7th Intern. Botan. Congr. Stockholm* pp. 34-42.
- Waid, J. S., and M. J. Woodman. 1957. A non-destructive method of detecting diseases in wood. *Nature* **180**: 47.
- Waite, M. B. 1891. Results from recent investigations in pear blight. *Botan. Gaz.* **16**: 259.
- Walker, J. C., and M. A. Stahmann. 1955. Chemical nature of disease resistance in plants. *Ann. Rev. Plant Physiol.* **6**: 351-366.
- Walker, J. C., F. J. Le Beau, and G. S. Pound. 1945. Viruses associated with cabbage mosaic. *J. Agr. Research* **70**: 379-404.
- Watts Padwick, G. 1956. Losses caused by plant diseases in the colonies. *Phytopathol. Papers* **1**: 60 pp.
- Weindling, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology* **22**: 837-845.
- Wellman, F. L. 1953. Some important diseases of coffee. Plant diseases. *U. S. Dept. Agr. Yearbook* **1953**: 891-896.
- Winter, A. G., and L. Willeke. 1951. Untersuchungen über Antibiotica aus höheren Pflanzen und ihre Bedeutung für die Bodenmikrobiologie und Pflanzensoziologie. *Naturwissenschaften* **38**: 262-264.
- Wood, J. I. 1953. Three billion dollars a year. Plant diseases. *U. S. Dept. Agr. Yearbook* **1953**: 1-9.
- Yarwood, C. E. 1957. Effect of rust infection on photosynthesis of bean. "Proceedings 4th International Congress of Crop Protection." Hamburg (in press).

## CHAPTER 3

# History of Plant Pathology

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Some of man's earliest known writings make reference to the ravages of plant diseases. In the Old Testament, blasting, mildew, and insect pests are included with human diseases and war among the great

scourges of mankind (Gen. 41:23; Deut. 28:22; I Kings 8:37; II Chron. 6:28; Amos 4:9; Hag. 2:17). Fossil fungi thought to be nearly 2,000,000,000 years old have been found in pre-Cambrian cherts (Tyler and Barghoorn, 1954). Seward (1931) states that "from the Devonian period onward and even from a more remote age there were parasitic and saprophytic fungi." There can be little doubt that plant diseases long preceded man on earth and that, as he developed a substantial agriculture far back of recorded history, they took their toll of his crops.

## I. THE BEGINNINGS OF BOTANY

Theophrastus of Eresus, who lived from 370 to about 286 B.C., was the first great botanist of whom convincing records are known (Greene, 1910; Hort translation, 1916). With Aristotle, he was a student of Plato. Later, he was a student and junior colleague of Aristotle, who bequeathed him his extensive library and botanic garden at Athens. Theophrastus conducted a school of about 2000 students. Although he compassed the whole field of learning of his day, he devoted much time and interest to botany. He is reported to have written 227 treatises, of which about one-twentieth dealt with botany. He brought together and greatly extended the botanical knowledge of his time. His "*Historia plantarum*" has been reviewed by Greene, who lists 19 outstanding contributions. The following excerpts from Greene's review illustrate their scope and importance:

"He distinguished the external organs of plants, naming and discussing them in regular sequence from root to fruit. . . . He classified such organs as (a) permanent, and (b) transient. . . . He divided the plant world into the two subkingdoms of the flowering and the flowerless. . . . The subkingdom of the flowering he again saw to be made up of plants leafy-flowered and capillary-flowered; really the distinction between the petaliferous and the apetalous. . . . He indicated the still more important differences of the hypogynous, perigynous, and epigynous insertion of corolla and androecium. . . . He distinguished between the centripetal and centrifugal in inflorescences. . . . He was the first to use the term fruit in the technical sense, as applying to every form and phase of seed encasement, seed included; and gave to carpology the term pericarp. . . . He classified all seed plants as (a) angiosperms and (b) gymnosperms. . . . He classified all plants as tree, shrub, half-shrub, and herb. . . . Theophrastus, with natural vision, unaided by as much as the simplest lens, and without having seen a vegetable cell yet distinguished clearly between parenchymatous and prosenchymatous tissues; even correctly relating the distribution of each to the fabrics of pith, bark, wood, leaves, flowers and fruit. . . . This list of facts which Theophrastus

saw, and in the main discovered, is not complete, but it embraces well-nigh all the first rudiments of what even today is universal scientific botany." This work is the foundation for Theophrastus' great reputation as the "Father of Botany."

In dealing with trees, cereals, and pulses, Theophrastus wrote briefly of their diseases. His approach was observational, deductive, and speculative, rather than experimental and inductive. In his treatment of plant diseases, he appears to have relied heavily on observations made by others, and some widely held erroneous beliefs of his time were passed on without challenge. The result was a mixture of keen observations and logical deductions with numerous errors of observation and interpretation. The following quotations from the Hort translation are illustrative:

Under the heading "Of diseases of cereals and pulses and of hurtful winds," he wrote: "As to diseases of seeds—some are common to all, as rust, some are peculiar to certain kinds; thus chick-pea is alone subject to rot and to being eaten by caterpillars and by spiders; and some seeds are eaten by other small creatures. Some again are liable to canker and mildew, as cummin. *But creatures which do not come from the plant itself but from without* [italics added] do not do so much harm; thus the *kantharis* is a visitor among wheat, the *phalangion* in vetches, and other pests in other crops.

"Generally speaking, cereals are more liable to rust than pulses, and among these barley is more liable to it than wheat; while of barleys some kinds are more liable than others, and most of all, it may be said, the kind called 'Achilleian.' Moreover, the position and the character of the land make no small difference in this respect; for lands which are exposed to the wind and elevated are not liable to rust, or less so, while those that lie low and are not exposed to wind are more so. And rust occurs chiefly at the full moon. . . ."

Theophrastus' views on spontaneous generation are of special interest. In his "De causis plantarum," he stated (see Theophrastus, Dengler translation, 1927): "Seed, it is agreed, may be secured from all plants in general; but because it is not used by husbandmen in the case of some plants, since they grow more quickly of their own accord, and because it is difficult in some plants, be they woody or herbaceous, to get the seed, there are some who think that not all plants come from seed. As was said, however, in the *Historia*, the possibility of such growth is quite evident even in figs. In still other manners do plants come into being, as for example: spontaneously, that is by conflux and decay, or even by more natural change that takes place [transmutation].

"Now it is clear that reproduction from seed is common to all plants. If some of them have both ways of reproduction; namely, by spon-



taneous generation as well as from seed, that is a marvel no greater than that of some animals, which produce either from their own kind or out of the earth.

"Spontaneous generation, to put the matter simply, takes place in smaller plants, especially those that are annuals and herbaceous. But still it occasionally occurs too in larger plants whenever there is rainy weather or some peculiar condition of air or soil. . . .

"The rains also produce certain decompositions and changes—when the moisture penetrates deeply—and they can nourish and increase the resulting growths under the warming and drying influence of the sun. . . ."

Theophrastus' work on plant diseases, as recorded in his "*Historia plantarum*," appears to have been minor and far inferior in quality to his contributions to phases of botany that were more approachable by the methods and the learning of his time. However, it reflects the state of knowledge of his day. Plant diseases caused great damage and were of very serious concern. Philosophers speculated on their causes and the people appealed to the gods for protection. There appears to have been no inkling of the true causes of infectious diseases. On the contrary, it was generally accepted that fungi and various other organisms observed in association with diseased or decaying plants arose spontaneously from the plants or from the environment. Supported by the authority of the great Greek philosophers, the erroneous belief that fungi associated with plant disease were a result, rather than an inciting cause, of disease dominated thought on this subject for more than 2000 years and greatly retarded the advancement of knowledge of infectious diseases.

## II. TWO MILLENNIA OF WAITING

Comparatively little was added to the knowledge of botany or plant pathology for nearly 2000 years after the time of Theophrastus. Greene (1910) has reviewed the botanical writings of the Greeks and Romans after Theophrastus. There were many writers in the period after Theophrastus to the 6th century A.D., but few thereafter for a thousand years. The botanical writings of this period, which were oriented largely toward medicine and agriculture, derived chiefly from Theophrastus and from the fragments that were added to his work.

References to plant diseases continued to attest to their importance, but added nothing substantial regarding their nature, cause, or control. Among the more noted Roman writers were Marcus Terentius Varro (117–27 B.C.), who in his "*De re rustica*" referred to Robigus as the god to be propitiated for protection against rust, and Caius Plinius Secundus (Pliny the Elder, 23–79 A.D.), who in his monumental compilation "*His-*

toria naturalis" included sections on plant diseases that contain virtually all that Theophrastus wrote on these subjects, with fragments from other sources.

From the 5th century A.D. until the Renaissance there was a paucity of intellectual activity, and little was written on botany or plant pathology. Writing about plant pathology of that period, Whetzel (1918) states that one bright spot in the all but universal darkness was the work in the 10th century of an Arabian landed proprietor, Ibn-al-Awam, who was said to have described accurately the symptoms of many diseases of trees and of the vine and to have given extensive consideration to their control. The references cited by Whetzel have not been available to the present writer.

### III. THE RENAISSANCE, THE DISCOVERY OF THE WESTERN HEMISPHERE, AND THE BEGINNINGS OF MODERN BOTANY

Development of modern plant pathology had to await a sufficient foundation in botany and other sciences from which it derives. This, in turn, had to await a change in intellectual climate that would make it possible to break the bonds of scholasticism, dogma, and inertia and to initiate free inquiry into natural phenomena. Beginnings of the intellectual awakening of the Renaissance in Europe in the 14th century heralded this change. As the Renaissance progressed, there was a general quickening of activities in all fields. Printing, which had been introduced into Europe in the middle of the 15th century, greatly facilitated learning, and the discovery of the Western Hemisphere several decades later opened prospects that fired men's imagination and spurred their activity. Science shared with the arts, literature, and commerce in a great forward movement and Christian religion underwent the Reformation. Although release from authoritarianism came slowly and painfully, the movement was on its way and the beginnings of modern science were in the making.

The beginning of a revival of interest in botany is reflected in the writings of the "herbalists." Greene (1910) has given an excellent account of the lives and works of four 16th century "German Fathers" of this revival: Otto Brunfelsius, Leonhardus Fuchsius, Hieronymus Tragus (or Bock), and Valerius Cordus. The first three named were clergymen and all were doctors of medicine. Cordus was a brilliant young scholar who died at the age of 29 on a botanical expedition. Greene states: "Brunfels and Fuchs busied themselves almost wholly with medical botany. It is a rare thing with either of them to mention a plant of unknown or even uncertain medical or alimentary qualities; and their plant descriptions are almost as uniformly either compiled or literally copied from authors

of centuries or even almost thousands of years before them. The books of Tragus and Cordus abound in new and original descriptions. These demonstrate that these two men examined plants with their own eyes, and for the love of them as plants, and they saw many things about the structure and the behavior of them to which the other two men, and even all botanists before them, had been blind." This 16th century work, which was done largely in response to the urge of medicine, aroused a new interest in botany and added to the foundations for the great movement in description, naming, and classification of plants that was to be the first step in the development of modern botany.

The development of modern science received a stimulus from the writings of Sir Francis Bacon. Although not himself an active scientist, Bacon (1605) clearly saw the limitations of the primarily deductive and speculative methods of the Greeks and Romans and of the scholasticism and authoritarianism that had enveloped and bound learning during the Dark and Middle Ages. He called vigorously for the use of inductive methods and for the development of institutions and facilities for free inquiry and for the advancement of learning in all fields (cf. Weld, 1848).

The great pioneering discoveries of modern science were largely the work of amateurs, who also took a major part in developing one of the great aids to science, the scientific societies. One of the oldest and most important of these is The Royal Society of London. An outgrowth of informal meetings of men with common scientific interests and enthusiasms, beginning about 1645, the Royal Society of London was more formally organized in 1660 and received its first charter from Charles II in 1662 (Weld, 1848). Communication to this Society was one of the most important channels for reporting the great discoveries that set modern science on its course.

Although amateurs played a major role in making these great early discoveries, they did not do this single-handed. Their contributions were made possible, in large part, by the legacies from scholars of antiquity, by the custodianship of learning by the Church in the Dark and Middle Ages, and by the rise of the universities in Europe.

The development of optical apparatus in the 17th century opened a new era in science. With extended vision, Galileo could look outward through his telescope and reveal a new cosmos and the early microscopists could look inward and discover a new world of the "infinitely small." Robert Hooke (1665) improved the compound microscope and saw that sections of cork and of other plant tissues were made up of minute units that he named cells. Marcello Malpighi (1675-79) of Italy and Nehemiah Grew (1682) of England, both physicians and amateur microscop-

ists, independently and virtually simultaneously laid the foundations of minute anatomy of plants so well that their work was not surpassed for a hundred years. Antony van Leeuwenhoek, a linen draper, surveyor, wine-gauger, and Chamberlain to the Sheriffs of Delft, Holland, ground the finest simple lenses of his time as an avocation, discovered bacteria (in 1675-76) and many other microorganisms, and opened up a great new science of microbiology (Dobell, 1932). All these men, except Galileo, reported their findings to the Royal Society of London, of which Hooke was Curator and later Secretary. Stephen Hales (1727), an English clergyman and a Fellow of the Royal Society, laid a foundation for modern plant physiology with his "Vegetable Staticks." He was a careful experimenter who studied especially sap movement and plant nutrition. While these new fields were being pioneered, the chief botanical activity continued to be describing, naming, and classifying plants. The pioneering stages of this movement culminated in the work of Carolus Linnaeus (1753) who, drawing heavily on the work of his predecessors, established the modern binomial system for plants and animals. The son of a clergyman, Linnaeus studied theology but turned to medicine and botany. He was Professor of Botany at the University of Uppsala from 1741 to 1778. His genius was for description and classification of higher plants, and his work is the starting point for nomenclature of this group. Great as his contribution was to taxonomy, Linnaeus accepted the doctrines of special creation and fixity of species. Along with Hooke, Grew, Hales, and most of the naturalists of his time, he believed with the ancients that small organisms could arise *de novo*.

As the foundations of modern botany were laid, similar advances were made in other fields of science, and at last the time was ripe for the beginnings of a modern science of plant pathology.

#### IV. THE DEVELOPMENT OF THE GERM THEORY OF DISEASE IN PLANTS

Knowledge of causation is the key to understanding disease. Knowledge of the pathogenic role of microorganisms is a key to understanding both infectious and noninfectious diseases, since it is essential for separating the effects of pathogens from those of the environment. Therefore, the establishment of the concept that microorganisms can incite disease in plants became the primary foundation of plant pathology, the various branches of which are largely outgrowths from this fundamental thesis.

##### *A. Experimental Proof of Reproduction in Fungi*

The first great step toward establishment of the germ theory of disease, after the development of the microscope, was the experimental



proof that fungi are autonomous organisms that reproduce by means of seedlike bodies, rather than capricious products of spontaneous generation. Buller (1915) has given a valuable account of this important advance.

Porta (1588) saw the black spore dust of mushrooms and stated that it was their seed, but he had no proof. Robert Hooke (1665) saw and figured teliospores of a *Phragmidium* taken from yellow spots on leaves. Buller states that this is the first illustration of reproductive bodies of a fungus. Hooke, however, thought these spores were seed pods rather than seeds. He believed that the fungus initially arose spontaneously but might produce seeds for its further propagation. Malpighi (1675-1679) also figured fungus spores, but regarded them as florets of an inflorescence, rather than as seeds. He thought, but did not prove, that fungi grew from seeds or fragments of themselves, rather than that they arose by spontaneous generation. Joseph Pitton de Tournefort (1705), a prominent French botanist, confidently expressed the view that fungi could reproduce by means of eggs or seeds and cause the dangerous moldiness disease of plants in humid greenhouses in winter. He thought humidity hatched the fungus eggs or seeds in minute crevices in plant surfaces, much as happens in moldiness of leather in cellars. He recommended keeping greenhouses drier to prevent moldiness. Thus, he clearly foresaw first, that fungi are autonomous organisms, rather than capricious creatures of spontaneous generation, and second, that they can incite disease in plants. However, he lacked the proofs, which Micheli was later to produce for the first great proposition and Prévost for the second.

Pier' Antonio Micheli (1729) was born in Florence, Italy, in 1679 of parents of limited means. Largely self-educated, he acquired an extensive knowledge of plants and was appointed botanist to the Grand Duke of Tuscany and placed in charge of the public gardens of Florence. His major work, "Nova Plantarum Genera," published in 1729 with financial aid of patrons, including Hans Sloane, President of the Royal Society of London, dealt mainly with higher plants but included his work on fungi, for which he is chiefly remembered. Micheli studied many fungi microscopically and identified their "seeds." He conducted an ingenious series of experiments with species of agarics, *Mucor*, *Botrytis*, and *Aspergillus* to test the reproductive ability of their "seeds." He scattered "seeds" of the agarics on dead leaves which he incubated on selected sites in the woods, and cultured those of the other fungi on freshly cut surfaces of melons, quinces, and pears. He varied the environmental conditions of the experiments, made replications and repetitions, and provided non-inoculated controls. The experiments with fruits gave clear-cut and con-



vincing results. The "seeds" consistently produced crops of their own kind. He attributed the few aberrant growths on seeded or control surfaces to air-borne spores that chanced to fall there. He figured spore clouds arising from *Lycoperdon* and *Fungoides* (= *Peziza*) and clearly understood that spores floated in air. Thus, he took one of the first great steps toward the overthrow of the theory of spontaneous generation; it remained for Pasteur nearly a century and a half later to take the last. A modest and devoted scholar, Micheli died unmarried at the age of 57 of an illness contracted on a botanical expedition. Although his great contribution to mycology and microbiology was neither appreciated nor widely accepted in his time, he was famous as a botanist and beloved as a man. It is fitting that he was interred in the Church of Santa Croce, where lie the remains of many of Italy's great sons.

### *B. Experimental Proof That Bunt of Wheat Is Contagious*

The next major advance toward establishment of the germ theory of disease was Tillet's (1755) experimental proof that wheat bunt is contagious and that it can be partly prevented by seed treatments. Mathieu Tillet, an amateur experimenter, was born in Bordeaux, France, about 1714. Little is known of his early life or education. For a time he was Director of the Mint at Troyes. In well replicated and controlled plot experiments over a period of three years, he proved conclusively that application of the black dust from bunted wheat to seeds from bunt-free plants greatly increased bunt in the crop they produced and that certain seed treatments, especially with a saltpeter solution and lime, partly prevented the disease. He did not realize, however, that bunt is incited by an organism. He thought the black dust contained a poisonous principle that could be partly antidoted. The brilliant design of his plot experiments would be highly creditable today. He was awarded a prize by the Royal Academy of Literature, Science, and Arts of Bordeaux for his Dissertation.

Tessier (1783), a prominent French agriculturist, repeated some of Tillet's experiments on bunt of wheat and conducted others of his own. He also studied several other diseases of cereals. He confirmed Tillet's results on the contagious nature of bunt. He reported that the bunt dust placed on the "germs" of wheat seeds resulted in a greater number of diseased plants than when placed on other parts of the seeds. He thought that treating seed wheat with extracts of bunt dust increased bunt. He conducted many seed treatment tests for prevention of bunt, using chiefly lime, alone in water or with various materials added. He also employed several mechanical methods for partial cleansing of the seed, including washing with water. His results, which were carefully com-

piled, indicated partial control from many treatments, but no sure and satisfactory control. He thought that reduction of bunt was due to reaction of the lime with the oily portion of the bunt dust. He did not recognize the parasitic nature of bunt, and concluded that the cause of the disease was unknown. He thought that soil moisture played a major role in the development of ergot of rye and that mists seemed to be the cause of rust of wheat, probably because of checking transpiration.

### *C. A Period of Classification of Plant Diseases and Speculation on Their Causes*

With the Revival of Learning, the budding science of plant pathology, like botany and zoology, entered a long period dominated by observation, description, classification, and speculation. The widely accepted concept of spontaneous generation and the dogmas of special creation and fixity of species were firm barriers to progress. There was also a strong tendency to distort descriptions and classifications to conform with the theories and terminology of medicine. Because of ignorance of the nature and causes of infectious diseases, confusion was compounded and the first great advances toward establishing the germ theory of disease were nearly lost.

A few writers who participated in this movement, however, did not accept the idea of spontaneous generation. De Tournefort (1705) was one of these. He divided plant diseases into two classes, the first due to internal causes and the second to external causes. He listed as internal causes too much sap, too little sap, bad qualities acquired by sap, and unequal distribution of sap to different parts of plants. Special interest attaches to his class of external causes, in which he included hail, frost, moldiness, plants hatched on other plants, insect injuries, and wounds. A clear-cut category of parasitic diseases was introduced here for the first time, but without adequate experimental foundation.

Hales (1727), writing of hops, stated that "... stagnating sap corrupts, and breeds moldy fen, which often spoils vast quantities of flourishing hop-grounds." He thought the "seed" of the mold might cause infection of the hops in successive years.

The prominent French botanist, Adanson (1763), followed de Tournefort in classifying plant diseases in two main groups, one attributed to internal causes and the other to external causes. He thought that mildew, rust, and smut diseases were caused by impeded transpiration, and regarded the associated fungi as products of the plant sap. He thought that the black dust of bunt, which he likened to the powder of *Lycoperdon*, was a secondary and perpetuating cause of this disease.

In 1766, grain rust occurred with great severity in Italy and was studied independently by two distinguished Italian scientists, Felice Fontana (1767), a brilliant professor of physical and biological sciences, and Giovanni Targioni-Tozzetti (1767), a prominent physician and botanist, who was the successor of Micheli. On the basis of careful microscopic examinations, both of these men concluded that the cereal rust diseases were caused by microscopic parasitic plants. Although their observations and interpretations were remarkably advanced for the time, experimental proof of their thesis was lacking.

In 1773, John Baptiste Zallinger (Sorauer, 1909), a professor of natural history at Innsbruck, Austria, went to an extreme in attempting to follow medical concepts and terminology. He was strongly of the opinion that fungi associated with plant diseases are products of the diseased plants, rather than causes of the disease.

Johann Christian Fabricius (1774), a Danish professor and a devoted student of Linnaeus, placed plant diseases in an elaborate system of classes, genera, and species. Probably because of the influence of Linnaeus, he was equivocal and far less advanced than de Tournefort and his followers, with regard to fungi as causes of plant diseases. He expressed the belief that the cause of rust and smut of cereals "is one and the same." After calling attention to the belief of Linnaeus that black smut powder soaked in water for some days turns into small worms which are the true cause of smut, he states: "A kind of movement is always observable when the black powder has been saturated; whether this is due to something animal, to something organic, or whether indeed it is the cause and not the effect of smut, is not absolutely certain. However, certain it is that the causes and symptoms of smut can never be better explained than by assuming something organized to be the cause."

The last major attempt to classify plant diseases without knowledge of the true causal role of microorganisms was made by Filippo Ré (1807), Professor of Botany and Agriculture at the University of Modena, Italy, in a treatise on diseases of plants. He divided plant diseases into classes and genera according to symptoms and supposed causes. Like so many of his predecessors, he was much influenced by medical concepts and terminology. However, he included a class of "indeterminate diseases." Of this class, in which he placed the rust and smut diseases, he wrote: "I have thus designated those diseases whose origin is either entirely unknown, or deduced from observations contradictory in themselves, or from hypotheses which, however brilliant, have no real foundation." After this excellent statement, he discusses the opinions of several prom-

inent writers on the cause of rust diseases and adds his own, which he summarizes as follows: "All this would lead me to lay down that the cryptogamic plants, the minute insects, or the exudations, whether dry or not, are rather symptoms of the disease itself, which is a result of excessive vigour or over-repletion."

#### *D. Early Development of Mycology*

The development of mycology was highly essential to progress in plant pathology. Beginning with the work of Linnaeus (1753), mycology entered a dominantly taxonomic period. Although Linnaeus worked but little with fungi, he made an important contribution to mycology by including them in his Latin binomial system. His work is the starting point for modern nomenclature of the Myxomycetes and lichens. Pierre Bulliard (Bulliard and Ventenat, 1809–12), a talented French botanist and mycologist, wrote a major book on mycology, "*Histoire des champignons*." This work was published in part, beginning in 1791; the second tome was completed by Ventenat after Bulliard's death and the complete work was published in 1809–12. Bulliard recognized four orders based on the position in which the "seeds" were borne. He used colored illustrations, which he prepared. The foundations of modern classification of fungi were laid chiefly by Persoon and Fries. Christiaan Hendrik Persoon, a native of South Africa, was educated in Germany and Holland and did most of his work in France. Although a doctor of medicine, he devoted most of his time to the study of fungi. His "*Synopsis methodica fungorum*" (1801) is the chief basis for all later classifications of fungi and is the starting point for the nomenclature of the Uredinales, Ustilaginales, and Gasteromycetes. He thought that some fungi arose spontaneously and that some grew from spores. He regarded smut fungi as products of the diseased plants. Elias Magnus Fries, son of a Swedish clergyman, was Professor of Botany, first at the University of Lund and later at Uppsala, where he succeeded Linnaeus. His monumental "*Systema mycologicum*" (1821–32) was designed to include all the fungi then known. It is the starting point for the nomenclature of all groups of fungi except those that start with the work of Linnaeus or Persoon. Fries regarded the rust and the smut fungi as products of the diseased plants. Among many other prominent early mycologists were Nees von Esenbeck (1816–17) of Germany, von Schweinitz of the United States of America (1822), Lévillé (1837, 1846, 1851) of France, Corda (1837–54) of Bohemia, and Berkeley (1857, 1860) of England. Although early concentration on taxonomy undoubtedly delayed progress in other aspects of mycology, it furnished an essential basis for further development of the science.



*E. Experimental Proof That Bunt of Wheat Is Incited by a Fungus  
and Can Be Controlled by a Fungicide*

In the same year in which Tillet published his experimental proof that bunt of wheat is contagious, a child was born who, a half century later, was to furnish brilliant proof of the cause of this classical disease and a sure method for its prevention. Born in Geneva, Switzerland, August 7, 1755, Isaac-Bénédict Prévost (Prévost, P., 1820) came of an old intellectual family. His early education was very irregular. At the age of 22, after trying two apprenticeships, he became the tutor of the sons of M. Delmas of Montauban, Département du Lot, France. After 2 years of intensive self-education at Montauban, he announced the intention of devoting himself entirely to his studies. His early interest was chiefly in mathematics; physics and natural history dominated later. At Montauban he was affiliated with l'Académie du Lot, of which he was a founder. He was elected to several other learned societies in France and Switzerland and was friend and correspondent of many of their distinguished members. In 1810 he accepted the chair of philosophy at the newly founded Faculté de Théologie protestante at Montauban. He filled this position with distinction until his death at the home of M. Delmas on June 10, 1819.

Bénédict Prévost published more than a score of papers on physics, chemistry, biology, and philosophy. His most important contribution was his "Mémoire sur la cause immédiate de la carie ou charbon des blés, et de plusieurs autres maladies des plantes, et sur les préservatifs de la carie" (1807), which was based on studies undertaken at the invitation of l'Académie du Lot. In this memoir, Prévost clearly showed "that the immediate cause of bunt is a plant of the genus of the uredos or of a very nearly related genus." As far as the present writer is aware, this work contains the first recorded adequate experimental proof and interpretation of the role of a microorganism in the causation of a disease. This discovery of the cause and a means of prevention of bunt of wheat gave to the world a key to understanding the causation and the prevention of all infectious diseases; it, therefore, ranks high among the great pioneering advances in science (American Phytopathological Society, 1956; Keitt, 1956).

Founded upon accurate and well controlled experimentation over a 10-year period, Prévost's essential findings stand unchanged. He gave an accurate and detailed description of the symptoms of bunt in its various macroscopic stages of development. He suspected and proceeded to prove that the "globules" in the bunted kernels were "gemmae" or spores of a cryptogam. He described and illustrated these spores in detail



and made extensive studies of their germination and of the development of the "bunt plant" in relation to time, temperature, substrata, toxic agents, age and previous treatment of the spores, and concentration of spores. Having concluded that he was dealing with a microscopic plant, he proceeded to prove by extensive inoculation experiments that it is the "immediate cause" of bunt and to ascertain conditions that favor or hinder infection. He pointed out "that the vegetation of this plant, as well as that of a majority of the uredos, begins in the open air and is completed in the interior of the plant that it attacks," suggesting for such organisms "the general denomination of internal parasitic plants." He observed germinated spores of the bunt organism in the soil and on the surface of wheat seedlings grown in infested soil. Although he did not succeed in observing the mode of penetration of the bunt fungus into the wheat plant or its growth to the wheat embryo, he correctly surmised that some ramifications of the bunt plant must penetrate into the very young wheat plant and later insinuate themselves into the embryo and fructify. He observed fructification in the embryo and germinated the spores. In extensive and refined toxicological studies, he found that certain copper salts, distilled water in which metallic copper had been left, and various other substances in solution would prevent germination of spores of the bunt fungus. He critically distinguished injurious and inhibitory from lethal effects and experimented extensively on relations of concentration of the toxic agent, time, and temperature to toxic effects. On the basis of this information and of his extensive knowledge of the disease, he experimented on prevention of bunt. He made suitably controlled field tests in which inoculated seed wheat was planted after having received various treatments. Spores of the bunt organism from treated seed were tested for germination, and data were taken on the development of the disease on the wheat grown in the field from the experimental seed. Excellent control of the disease was obtained by steeping the seed wheat in a copper sulfate solution, and detailed practical recommendations were made for large-scale seed treatment.

Prévost's work, which was remarkably comprehensive and well correlated, laid a firm foundation for nearly all branches of modern plant pathology. He developed methods for obtaining virtually pure cultures of spores of the bunt fungus and for keeping them free from contamination by air-borne reproductive bodies of other microorganisms. He expressed his disbelief in spontaneous generation. He regarded the bunt plant as the "immediate" or "direct," rather than the sole cause of bunt because he clearly proved that the fungus can incite the disease only under sufficiently favorable conditions. He thus recognized the conditioning or secondary causal relationship of environment.

Prévost's great contribution was rejected by the academicians whose views he had so brilliantly refuted. He was too far ahead of his time. The autogenetic theory of disease was dominant in places of authority. When his memoir was submitted to the Académie des Sciences at Paris for evaluation, it received an adverse report (Prévost, P., 1820). Prévost's work, however, was never entirely overlooked, and it undoubtedly had an important influence on later work. The memoir was well reviewed in at least two popular journals and the method of seed treatment became widely used without benefit of academic approval. The memoir was also cited in botanical literature, but chiefly from reviews, since the original was very inaccessible. Of special importance was the extensive reference made to it by the Tulasnes (1847), with every evidence of confidence in the work and acceptance of its essential conclusions. Such treatment by the leading mycologists of the time brought Prévost's work into conspicuous attention. Thereafter, he was widely cited for the dominantly mycological findings that they emphasized, but his major contribution continued to lack recognition. Nearly a half century had to elapse before the world was ready to accept Prévost's major thesis.

#### *F. Retarding Influence of the Autogenetists*

The autogenetic theory of disease continued to be dominant throughout the first half of the 19th century. Among its most influential proponents at this time were Unger, Meyen, and Liebig. Franz Joseph Andreas Nicholas Unger was an Austrian physician and an eminent professor of botany. Although primarily a plant physiologist, he devoted much study to plant diseases. His best-known work on plant pathology was his "Exantheme der Pflanzen" (1833). He thought that fungi associated with plant diseases arose from the diseased plant because of abnormalities in the plant juices, and were, therefore, products rather than causes of disease. Franz Julius Ferdinand Meyen was a physician and a brilliant young Professor of Botany at the University of Berlin. He wrote on many aspects of botany, especially physiology and anatomy. His most important work on plant pathology, "Pflanzen-Pathologie" (1841), was published a year after his untimely death at the age of 36. Firmly wedded to the autogenetic theory, he regarded fungi associated with plant diseases as pseudoorganisms which resulted from abnormal nutrition of the plants. Justus von Liebig (1853?a, b) was one of the most famous chemists of his time. A doctor of medicine, though not active as a physician or biologist, he was a very influential opponent of the germ theory of disease. His ideas about the nature of disease were based on chemical theory, rather than experiments with diseases. He held that fermentation, putrefaction, and contagious disease resulted

from an active state of atoms, and that this active state of the atoms of one body could be transferred to those of another body in contact with it. Evidently much influenced by Hales, whose work he reviewed at length, he attributed the onset of the "potato disease" to stagnation of the plant juices because of suppressed transpiration and believed that the fungus observed on the leaves and the rotting of the tubers were consequences of the death of the plants. An appendix reports a method proposed by Klotzsch (Keeper of the Royal Herbarium, Berlin), for preventing potato diseases. It consisted of pinching off one-half inch from all growing tips of potato branches at specified intervals. Liebig's great and deserved reputation as a chemist gave undue weight to his opinions concerning the cause of diseases. Thus, preconception, speculation, and subservience to authority hindered the progress of experimentation and open-minded interpretation.

### G. The "Potato Disease" and the Irish Famine

Devastating epidemics of the "potato disease" near the middle of the 19th century tragically dramatized the importance of plant diseases and greatly stimulated interest in plant pathology. Much of the early literature of this disease is found in the *Gardeners' Chronicle*. It has been discussed by Jones *et al.* (1912) and Large (1940). The potato, which was introduced into Europe from South America in the latter half of the 16th century, had become a major food crop in many countries. It was the main source of food for the majority of the people of Ireland. Between 1830 and 1840, the disease now known as late blight, incited by *Phytophthora infestans* (Mont.) DBy., appeared in Europe and the United States. In 1845 and 1846, epidemic outbreaks virtually destroyed the potato crops of Europe. Famine resulted in Ireland, where it is estimated that hundreds of thousands of people died because of direct or indirect effects of malnutrition. Such a great wave of emigration followed that the population of Ireland was reduced by more than one-fourth. A social repercussion of the famine was the repeal of the Corn Laws.

Scientists of the time were unable to agree on the cause of the disease or to propose a successful remedy. The autogenetic theory of disease was still dominant, although the opposition was rapidly increasing. Dr. John Lindley, Editor of the *Gardeners' Chronicle* and Professor of Botany at University College, London, was a leader of the autogenetists. Berkeley (1845, 1846, 1848), who was then the most prominent British mycologist, was at first somewhat reserved in supporting the parasitic theory, but came out unequivocally for it in 1846 and thereafter. Von Martius in Germany in 1842 appears to have been the first to describe the disease

and the associated fungus, which he thought was the cause. Montagne in France in 1845 described the fungus as *Botrytis infestans*. Morren in Belgium experimented with the disease in 1845. He described the disease and the fungus and regarded the fungus as the major cause of the malady, although he lacked convincing evidence. He recommended spraying the ground with a mixture of copper sulfate, table salt, and lime in water to prevent tuber rot. Unfortunately, he did not apply the mixture to the foliage as a preventive of blight. Many committees and commissions were formed to report on the disease and there was much controversy over the opposing autogenetic and parasitic theories of its cause. The causal role of the fungus was finally proven experimentally by Speerschnneider in 1857, and De Bary in 1861 and 1863 (Jones *et al.*, 1912). Not until the discovery of Bordeaux mixture, nearly 40 years after these great epidemics began, was an adequate control measure found. All this time, the neglected work of Prévost contained the key to the solution of the problem.

#### H. *The Foundation of Modern Mycology and Acceptance of the Concept That Fungi Can Incite Disease in Plants*

The Tulasne brothers in France and De Bary in Germany were the outstanding founders of modern mycology. Louis-René Tulasne was educated as a lawyer but preferred botany. In 1842 he was appointed *aide naturaliste* at the museum of the Jardin des Plantes at Paris, where he worked until his health failed in 1864. Charles Tulasne, who studied medicine in Paris, gave up a medical career to join in his brother's botanical work. Louis-René was the leader. Charles collaborated in the studies and prepared mycological illustrations which have never been surpassed in artistic quality and workmanship. The Tulasnes' most important work dealt with the rust and the smut fungi and the Ascomycetes (e.g., 1847, 1854, 1861-65). They made meticulous studies of species in various stages of development, and thus discovered polymorphism in fungi. Their work threw a great flood of light on the morphology and natural relationships of fungi and on their potentialities as pathogens. The Tulasnes (1847) referred most favorably to Prévost's work and accepted his concept that fungi can incite disease in plants. Their crowning work was the superbly illustrated "Selecta fungorum carpologia."

Heinrich Anton De Bary, born January 26, 1831, in Frankfurt-am-Main, Germany, received his M.D. degree from the University of Berlin in 1853. Preferring botany to medicine, he was appointed lecturer at Tübingen in 1853, where he was associated with von Mohl. In 1855 he



succeeded Nägeli at Freiburg, and in 1867 he was called to Halle. In 1872 he became Rector of the University of Strassburg, where he continued to develop his famous school until his death in 1888, in his 57th year.

Although mycology was central in De Bary's work, he contributed to many aspects of botany and was one of the most outstanding biologists of his time. His famous "*Untersuchungen über die Brandpilze*" (1853), published when he was only 22 years old, is credited with establishing beyond further serious opposition that fungi are causes and not results of plant disease. In the first two parts of this book he reported thorough microscopic studies of the structure and development of numerous smut and rust fungi, including their relationships to the tissues of the diseased plants, and discussed their systematic relationships and classification. In the third part, he dealt with the relationships of these fungi to the smut and rust diseases. After a thorough discussion of literature and of his own observations, he concluded: "It has been shown that the smut and rust fungi originate not from the cell content or from the secretion of diseased cells and that they are not the result, but the cause of pathological processes." He suggested destruction of diseased parts of plants as a method of preventing such diseases, but recognized that this could not always be done. "Hence," he wrote, "for agriculture a successful result will be obtained only by seeking in the main to prevent in every way the development of smut and rust fungi, and therefore as far as possible destroying their spores, the 'smut dust.' This seems to be accomplished by means of the various corrosives which the farmers use for disinfection of the seed . . . undoubtedly and indeed chiefly by means of copper sulfate and lime."

The foregoing quotation illustrates how far scholars were behind plant culturists in 1853 in the chemical control of plant diseases, which had vitally important implications that favored the parasitic theory. Empirical chemical treatments of seeds and plants were reported by Theophrastus and Pliny. Probably old in Theophrastus' time, such treatments were undoubtedly tried through the centuries, with little or no convincing evidence of success until Tillet (1755) was able to demonstrate partial control of bunt of wheat by liming and similar treatments. No reliable and effective chemical control of an infectious plant disease was established, however, until Prévost (1807) gave a fully rational demonstration and interpretation of control of bunt by treating the seed wheat with a solution of copper sulfate. Others had previously tried copper sulfate for bunt control, but had failed to prove its value (Prévost, 1807; Woolman and Humphrey, 1924). In the hands of plant



culturists, sulfur, which had been applied empirically to plants since ancient times, came into successful use in controlling surface mildews in the first half of the 19th century. John Robertson (1824), in Ireland in 1821, reported a careful study of peach mildew, in which he correctly interpreted the causal role of the fungus and successfully controlled the disease by repeated applications of a preparation of sulfur and soap in water, by means of a syringe. This treatment, or modifications of it, came into use by gardeners, and recommendations of preparations of lime and sulfur soon followed (Lodeman, 1896).

The first generally accepted method for chemical control of a major disease by treating plants in foliage was developed by gardeners and viticulturists. The powdery mildew of the vine was first observed in Europe in glasshouses at Margate, England, in 1845 by Edward Tucker, a gardener (Berkeley, 1847). After satisfying himself by microscopic study that he was dealing with a fungus similar to that of peach mildew, Tucker applied a preparation of sulfur and slaked lime in water to the diseased leaves by sponging or by rubbing it on with his hands. The mildew in his houses was controlled, while in the next garden it developed destructively. As the disease spread rapidly and threatened the vineyards of Europe, many modifications of the sulfur treatment were tried. According to Marès (1856), Gontier, a French gardener, obtained excellent results in controlling the mildew in glasshouses in 1850 by applying sulfur dust to moistened vines by means of a bellows. In 1851, another French gardener, Grison, reported successful control of the mildew by a diluted preparation made from sulfur and freshly slaked lime boiled in water (Heuzé, 1852). A commission reported favorably on his work, and his preparation "eau Grison," a precursor of modern lime-sulfur, was used by gardeners against surface mildews for many years. In 1852, a gardener in France, Bergman, reported successful control of grape mildew by moistening the hot water pipes of glasshouses and powdering them with sulfur (Truffaut, 1852). A commission reported favorably on his results. Marès (1856) states that decisive results were obtained in 1853 on 300 acres of vines at Thomery, France, by using a method proposed by R. Charmeux of dusting the dry vines with sulfur. With modifications, this method came rapidly into general use and saved the European vineyards.

When Berkeley (1847) named the powdery mildew fungus of the vine *Oidium tuckeri*, n.s., interpreted it as the cause of the disease, and reported Tucker's control method, he met little opposition (Large, 1940). Thus, when De Bary published in 1853, the battle against the auto-genetists had been largely won. De Bary himself actually contributed far

less convincing evidence in 1853 for the thesis that fungi can incite disease in plants than Prévost had presented in 1807. De Bary never saw Prévost's memoir, which was not available to him, and had only fragmentary information about it through reviews. His own work was entirely observational; he made no experiments for inducing or preventing disease. Like Prévost, he was unable to observe the penetration of a pathogen into plant tissues. If his "Untersuchungen" had been his only work, he might not have been remembered long. Although this early work was very important, his great and fully deserved reputation was earned chiefly by the superb contributions that he made throughout the remainder of his life. These greatly accelerated the development of mycology and plant pathology.

Many other advances of great biological significance were made in the early and middle 19th century. Among these were the synthesis of urea by Woehler in 1828, the discovery of the cell nucleus by Robert Brown in 1831, the founding of the cell theory by Schleiden and Schwann in 1838-1839, the contributions to plant embryology by Hofmeister in 1849 and 1851, the works of Wallace and Darwin on the origin of species in 1858 and 1859, and the discovery of the laws of heredity by Mendel (1866) (von Sachs, 1875).

#### V. SOME EFFECTS OF THE STEAM AGE ON PLANT DISEASES AND PLANT PATHOLOGY

The advent of the steam age at the beginning of the 19th century had profound effects on problems of plant disease. Availability of cheap power led quickly to industrialization, and steamships and railroads furnished the transportation that permitted rapidly increased urbanization. As the cities grew large, an extensive and intensive agriculture was necessary to support them. Small and diversified plantings were increasingly replaced or supplemented by large acreages devoted to single crops, and often to single varieties. Since incidence of infectious disease is a function of density of population, losses from plant diseases greatly increased and the need for increased study of plant pathology became urgent. Out of this need has grown the present highly organized science of plant pathology.

#### VI. ESTABLISHMENT OF MAJOR TRENDS OF WORK CENTERING ABOUT GROUPS OF CAUSAL AGENTS OF DISEASE

Trends of investigation naturally developed about groups of causal agents of disease. Since fungi were the first microorganisms shown to incite disease, the first great trend in plant pathology was mycological.

### A. The Mycological Trend

#### 1. De Bary and His School

De Bary and his school gave great impetus to the development of the mycological trend in plant pathology, from which the subsequent trends largely derived. He was a great teacher, investigator, compiler, and educator. Students came to his laboratory from many countries. More than 60 of them became prominent in their fields and carried his teachings and influence to many parts of the world. Among these were Woronin of Russia, Brefeld of Germany, Millardet of France, Ward of England, Farlow of the United States of America, and Fischer of Switzerland.

De Bary was especially interested in the morphology, physiology, parasitism, sexuality, and natural relationships of fungi. His compilations of knowledge in this field were of great value to mycology and plant pathology. His "Morphologie und Physiologie der Pilze, Flechten und Myxomyceten" (1866) was followed by his "Vergleichende Morphologie und Biologie der Pilze, Mycetozoen und Bakterien" (1884), a masterpiece which was for many years the leading source of reference in its field. "Vorlesungen über Bakterien" appeared in 1887.

Especially noteworthy among De Bary's more direct contributions to plant pathology were his studies on the Peronosporaceae (1881) and diseases they incite, especially the "potato disease" (1861, 1863, 1876), his discovery of heteroecism in the rusts (1866-67), and his work on *Sclerotinia sclerotiorum* (1886), which opened up a new field of investigation into the physiology of parasitism. In these and other works he abundantly developed the experimental element that was lacking in his first publication. Because of his own work and that of his students, the influence of De Bary will long be felt.

#### 2. Kühn and Economic Plant Pathology

Julius Gotthelf Kühn was born in Pulsnitz, Saxony, in 1825. He was trained as a farm manager. He managed large estates and began agricultural investigations which stimulated his interest in further scientific education. At the age of 30, he entered the Agricultural Academy at Poppelsdorf and a year later transferred to Leipzig, where he received his doctor's degree in 1856. After a term as lecturer at the Agricultural Academy at Proskau, he became manager of a large estate in Silesia; there he wrote his famous textbook on plant pathology. In 1862, at the age of 37, he was called to the newly created chair of agriculture at the University of Halle. He remained at Halle until near the time of

his death in 1910, and saw the fulfillment of his hopes of introducing agriculture into the University structure. His interests in agriculture were very broad. He was the author of more than 250 published papers, of which some 70 dealt with mycology or plant pathology.

Kühn's "Die Krankheiten der Kulturgewächse" (1858) was the first textbook on plant pathology written in the full light of knowledge of the role of fungi in the causation of plant diseases, although Berkeley (1854-57) had published a very valuable series of 173 articles on "Vegetable Pathology" in the *Gardeners' Chronicle*, beginning January 7, 1854, and ending October 3, 1857. With the rapidly accumulating knowledge of fungus diseases and with his great knowledge of agriculture, Kühn was able to bring his science to bear on the practical problems of plant disease and to recommend specific control measures.

Kühn listed as causes of plant diseases unfavorable climatic and soil conditions, animals (insects), phanerogamic parasites, and cryptogamic parasites. Special consideration was given to smut of cereals, rust of cereals and of legumes, ergot, mildew, sooty-mold and honey-dew, leaf blight or leaf spot diseases, disease of rape and rape seed, seed rot of Fuller's teasel, the gout or cockle disease of wheat, and diseases of tuber or root crops. Of special interest were his contributions on bunt of wheat. He discovered and figured direct penetration of the bunt fungus into the wheat seedling, traced its development to the bunted kernel, and standardized the copper sulfate seed treatment method which farmers had been using for a half century since Prévost had discovered it.

Kühn contributed greatly to the development of the economic as well as the scientific aspect of plant pathology and to the advancement of the study of agriculture to the university level.

### 3. Brefeld and the Development of Mycological Techniques

Born in Telgte, Germany, in 1839, Oskar Brefeld was educated as a pharmacist. Preferring botany, he studied under Hofmeister at Heidelberg and De Bary at Halle. After an appointment at the Forest Academy at Eberswalde, he held professorships successively at the Universities of Münster, Breslau, and Berlin and died in Berlin in 1925.

Brefeld (1875, 1881, 1883), working with fungi, was the leader in the early development of modern techniques for growing microorganisms in pure culture. With the refinements made by Koch, Petri, and others, his techniques are the foundation for the pure culture methods currently employed. After his earlier studies on the complete life cycles of saprophytic fungi, he gave major attention over a period of some 30 years to the smut fungi and diseases (1888, 1912; Brefeld and Falck, 1905). He was the leader in tracing the life cycles of the cereal smut



fungi and their role in the causation of diseases. In this work he made important contributions both to pure culture of smut fungi and to inoculation techniques.

#### 4. *Hartig and the Foundation of Forest Pathology*

Heinrich Julius Adolph Robert Hartig was of the third generation of a distinguished line of foresters. His grandfather, Georg Ludwig Hartig, was chief forester of Prussia. Theodor, his father, a famous botanist and forester, was Professor of Forestry at the University of Berlin. Robert was born in Braunschweig, Germany, in 1839. After training in forestry, he studied at the Universities of Berlin and Marburg, and obtained his doctor's degree from the latter in 1867. His most important work was done at the University of Munich, where he was Professor of Botany and Director of the Royal Forestry Experiment Station.

Robert Hartig was an enthusiastic and successful teacher, an accurate, thorough, and productive investigator, and a voluminous writer. A man of broad interests and great energy, he made valuable contributions to many aspects of forestry, botany, and entomology. He is widely known as the "Father of Forest Pathology" because of his great pioneering work in this field. Outstanding among his many contributions to forest pathology were his "Wichtige Krankheiten der Waldbäume" (1874) and his "Lehrbuch der Baumkrankheiten" (1882). The latter was for many years the most comprehensive and authoritative work in its field.

#### 5. *Millardet and Bordeaux Mixture; Stimulus to Research in Biology and Agriculture*

Pierre Marie Alexis Millardet was born in Montmery-la-Ville, France, in 1838. He began the study of medicine in Paris, but was more interested in botany, which he studied under Hofmeister at Heidelberg and De Bary at Freiburg. He returned to France and took his doctorate in both medicine and science. After appointments at the Universities of Strasbourg and Nancy, he was called in 1876 to the chair of botany at the University of Bordeaux, where he served until he retired in 1899.

Millardet was an able and imaginative investigator with broad interests. His works may be divided into three major groups: (1) early studies on the morphology, physiology, and systematic relationships of plants; (2) investigations of Phylloxera of the vine, including introduction of resistant stocks from North America and extensive hybridization experiments aimed at obtaining resistant varieties and understocks; (3) researches on diseases of the vine, especially the downy mildew and its control by Bordeaux mixture. In these investigations, Millardet became a pioneer in the development of two of the most important means of plant



disease control—use of disease resistant plants (1878, 1891a, b, 1894) and application of fungicidal sprays to plants in foliage (1885a, b, c; Lodeman, 1896; Large, 1940).

The downy mildew of the vine was first reported in Europe in 1878; Millardet and Planchon found it at about the same time in France, where it evidently had been introduced from the United States of America. The disease spread rapidly and threatened to ruin the vineyards of Europe. Millardet promptly began a thorough study of the disease and its control. In October of 1882 he noticed that vines that had been treated with a mixture of copper sulfate and slaked lime to deter pilferers retained their leaves, whereas the untreated vines were defoliated. In 1883 and 1884 he performed extensive spraying experiments with many preparations of copper, calcium, and iron salts, used alone and in various mixtures; he also arranged tests by viticulturists. Both seasons were dry and little mildew developed. Being a conservative scientist, Millardet preferred to delay publication until he could recommend a thoroughly tested spraying program. However, the news of his work spread and others began to publish the effects of copper preparations on mildew. In May, 1885, Millardet published his work and gave detailed recommendations for spraying with a mixture of copper sulfate and slaked lime, later known as "Bordeaux mixture." Mildew was severe in 1885 and Millardet's recommendations were followed extensively, with spectacular success. He and others rapidly improved spraying methods and studied a great variety of copper and other fungicidal preparations. Bordeaux mixture emerged as the most successful one for a long period until the need for fungicides less injurious to some host plants was clearly recognized.

Mastery of the mildew and saving the vineyards of Europe was a spectacular accomplishment but only a foretaste of what was to come. As experimenters in many countries eagerly joined in the investigations, Bordeaux mixture began a triumphant march around the world. The dreaded "potato disease" and one after another of the major plant diseases toppled before it. Never before had there been such a dramatic, world-wide demonstration of what science could do for agriculture. The discovery of Bordeaux mixture gave a great stimulus to the development of agricultural institutions, and more broadly, to increased study of science and its relation to human affairs.

## 6. *Fungus Diseases of Plants Studied Around the World*

In the latter half of the 19th century plant pathology was concerned chiefly with exploiting the great fundamental concepts experimentally founded by Prévost and confirmed and extended by the Tulasnes, De Bary, and others. Fungus diseases, the most important and best known

group, were studied by many able investigators around the world. The knowledge and the methods derived from their study resulted in increasing recognition of other groups of causal agents of plant disease. Around these new or more clearly recognized groups, arose new trends of work.

It is noteworthy that the chief principles and most of the basic methods of plant disease control were discovered in the development of the mycological trend in plant pathology. The beginnings of fungicidal control of plant diseases have been noted on earlier pages. Both Prévost and De Bary clearly recognized the principle of eradication of pathogens for plant disease control and proposed specific eradicated procedures. Their work and similar investigations which followed provided the rationale for methods of eradication and exclusion of pathogens, which were extensively developed in the latter half of the 19th century. Similarly, establishment of the fact that fungi can incite disease in plants gave a rational basis for selecting and breeding plants for disease resistance. Comparatively little progress was made along this line, however, until the rediscovery of Mendel's laws of heredity gave a scientific foundation for plant breeding.

Theophrastus had noted that some plants are less liable to disease than others, a fact that probably was known long before his time. Consciously or unconsciously, plant culturists through the ages have undoubtedly accelerated natural breeding and selection for disease resistance by propagating from plants that seemed superior for their purposes. The great epidemics of the "potato disease" stimulated efforts in the latter half of the nineteenth century to breed blight resistant potato varieties. Limited success was obtained from crosses of South American stocks with American and European varieties (Jones *et al.*, 1912). Millardet (1878; 1891a, b; 1894) made extensive crosses of European and American varieties of grape in an effort to develop varieties of the European type resistant to *Phylloxera* and downy mildew. He obtained *Phylloxera* resistant understocks on which European varieties could be successfully propagated. Farrer (1899), an Australian wheat breeder, initiated successful experiments to produce varieties resistant to stem rust.

The rediscovery of Mendel's laws in 1900 greatly stimulated breeding plants for disease resistance. Biffen (1905, 1907, 1912) was the first to report the application of this knowledge to inheritance of disease resistance. Working at Cambridge University, England, with stripe rust of wheat, incited by *Puccinia glumarum* (Schm.) Erikss. and Henn., he showed that in crosses of a resistant and a susceptible variety, resistance was inherited as a recessive Mendelian character. Beginning in 1900, Orton (1900, 1909), in the United States, made spectacular progress in the control of *Fusarium* wilts of cotton, watermelon, and cowpea by

selecting and breeding resistant varieties. The work of Biffen and Orton established breeding for disease resistance on a firm scientific and practical foundation.

### B. *The Physiological Trend*

The physiological trend in plant pathology may be regarded as having three major aspects: (1) studies of physiogenic diseases, (2) work on the indirect or secondary causal relationships of environment to infectious diseases, and (3) investigations into the physiology of parasitism. The first of these constitutes the separate and distinct physiological trend; the second and third are joint aspects of the physiological and other trends.

The relationships of environmental factors to the causation of disease could not be reliably determined until the causal role of microorganisms was known. As late as the middle of the 19th century, many leading scientists were still attributing fungus diseases to environmental influences. Kühn (1858), knowing the causal role of fungi, included in his book a reliably differentiated class of diseases caused by unfavorable climatic and soil conditions. Sorauer, however, was the leader in establishing modern studies of physiogenic diseases.

Born in Breslau, Germany, in 1839, Paul Karl Moritz Sorauer studied botany and received his doctor's degree at Rostock in 1867. His longest assignment was as Director of the Experiment Station for Plant Physiology at the Imperial Cider Institute of Proskau from 1872 to 1893, after which he retired because of an eye ailment. He continued to work, however, and was later Privat Docent at the University of Berlin. He was noted as a teacher, compiler, and editor and was a leader in efforts to develop international cooperation to limit the spread of plant diseases. He was a founder and for some 25 years the editor of *Zeitschrift für Pflanzenkrankheiten*. He died in 1916. His best known work was his "Handbuch der Pflanzenkrankheiten," the first edition of which was published in 1874. This book, which was immediately very successful, has been revised and expanded through six editions and is still widely used. Sorauer's chief interest was in physiogenic diseases, and he gave these emphasis and space comparable to that devoted to parasitic diseases.

Prévost (1807) demonstrated experimentally and interpreted clearly the indirect or secondary causal role of environment in the etiology of parasitic disease. Sorauer (1874) and Ward (1902b) were other outstanding pioneers in studying influences of environment on parasitic plant diseases. Both stressed the importance of predisposition of host plants to disease by environmental influences prior to infection.

De Bary (1886), in his last work, pioneered in the fertile field of physiology of parasitism. Working chiefly with *Sclerotinia sclerotiorum* and the disease it incites on carrots and other plants, he observed that host cells were killed in advance of the invading hyphae of the fungus. He expressed juice from rotted tissue and found that it could rapidly break down healthy host tissue. This activity of the juice was lost on boiling. De Bary thought that the fungus was a saprophyte which became facultatively parasitic by producing an enzyme or enzymes that killed plant cells and made their contents available for its nourishment.

Another eminent pioneer in studies of the physiology of parasitism was Ward. Harry Marshall Ward, one of England's greatest botanists, was born in 1854 and graduated from Cambridge in 1879. After a brief period of study in Germany under Sachs and De Bary, he was commissioned to investigate the rust disease of coffee, which was destroying the plantations of Ceylon. He made a thorough 2-year study of this disease, but was unable to save the plantations. After returning to England, he was for some years Professor of Botany at the Royal Engineering College at Cooper's Hill. In 1895 he was called to the chair of botany at Cambridge, where he rendered distinguished service until his death in 1906 at the age of 52. Plant pathology was his central interest, with special emphasis on host-parasite relationships. An outstanding contribution on the physiology of parasitism was his work on "A Lily-Disease" (1888) incited by a *Botrytis* species. He made a thorough study of the fungus and of its penetration and invasion of host tissues. He obtained from pure cultures of the fungus a highly purified "ferment" that would break down healthy host tissues. Although he could not find a perfect stage of his fungus, he surmised that it was a *Peziza* (*Sclerotinia*). He thought De Bary's *Sclerotinia sclerotiorum* was intermediate in its parasitism between his *Botrytis* and saprophytic pezizas and that it was in process of being "educated" to parasitic habits. Ward's belief that fungi can thus be educated was strengthened by his famous brome rust investigations (1902a, 1903). Although later work did not support his interpretation of "bridging hosts" in his rust work, nor his conclusion that secretions produced by *Botrytis* dissolve the plant cuticle and thus permit penetration by the fungus, these mistakes in interpretation of pioneering experiments on very difficult problems are rare exceptions in his brilliant career.

### C. The Bacteriological Trend

The concept that fungi can incite diseases in plants had been generally accepted for nearly a quarter of a century before a similar role was proved for bacteria. As it had been with fungus diseases, a great



stumbling block was the ancient concept of abiogenesis. In one of the most brilliant series of researches in the history of science, Pasteur finally completed the overthrow of the theory of spontaneous generation and paved the way for convincing experimental proof by Koch (1876) and himself that anthrax is incited by a bacterium. This great work has been adequately reviewed elsewhere (Ducleaux, 1896; Dubos, 1950). It revolutionized animal pathology and medicine and had wide implications for plant pathology, agriculture, industry, and other fields.

Burrill in the United States and Wakker in Holland were pioneers in producing the proof that bacteria can incite diseases in plants. Born in Massachusetts in 1839 and educated in Illinois, Thomas Jonathan Burrill was for many years Professor of Botany and Horticulture and Vice President of the University of Illinois. In studies of fire blight of pear and apple, chiefly from 1877 to 1883, he showed that a bacterial organism was constantly and abundantly present in freshly blighted tissues and that he could incite the disease consistently by direct inoculations (1878, 1881, 1884). J. H. Wakker (1883, 1889), a young botanist at the University of Amsterdam, began work on the yellow disease of hyacinth in 1881. He found bacteria abundantly in the diseased tissues and was able to incite the disease consistently by direct inoculation, beginning in 1882. Arthur (1885, 1887a, b) confirmed and extended Burrill's work on fire blight, inciting infection with pure cultures of the bacterium. Savastano (1887), in Italy, showed that olive knot is incited by a bacterium. Most of this early work on bacterial diseases of plants was done with methods and standards far below those developed by Koch and Pasteur in studies on bacterial diseases of animals. Many scientists, especially in Europe, still doubted that bacteria incited diseases in plants (Fischer, 1897, 1899), or thought that such diseases were unimportant (De Bary, 1884, 1887). The outstanding leader in bringing the best available methods and standards to the study of bacterial diseases of plants, and in establishing the modern bacteriological trend in plant pathology, was Smith.

Erwin Frink Smith, one of the greatest plant pathologists, was born in 1854 in the State of New York. He received his B.S. degree in 1886 and his Sc.D. in 1889 from the University of Michigan. His long and fruitful career was spent in the United States Department of Agriculture. He died in 1927 at the age of 73. His work on bacterial diseases of plants began with successive studies on wilt of cucurbits (1895), brown rot of solanaceous plants (1896), and black rot of cruciferous plants (1897). Outstanding among his later works are his "Bacteria in Relation to Plant Diseases" (1905-14) and his extensive researches on crown gall (e.g., Smith *et al.*, 1911, 1912). In his controversy with Fischer, he (1899a, b, 1901) silenced the last doubters of the occurrence or the importance of bacterial diseases of plants.



### D. *The Virological Trend*

Although diseases now known to be incited by viruses have been described in literature for at least several centuries, their etiology could not be determined until sufficient knowledge and techniques were developed to distinguish them from fungal and bacterial diseases. The first great step in discovery of the etiology of a virus disease of plants was the experimental transmission of tobacco mosaic by Mayer (1886). Adolf Mayer was born in Oldenburg, Germany, in 1843 and educated as a chemist at the Universities of Heidelberg, Ghent, and Halle. From 1876 to 1904, he was Director of the Agricultural Experiment Station at Wageningen, Holland; before and after this appointment he was a professor at Heidelberg. About 1880 he began work on a serious tobacco disease, which he named mosaic. He was able to incite the disease consistently by injecting juice from diseased leaves into healthy tobacco plants. The infectivity was not impaired when the juice was heated at 60° C., but was reported as lost when it was held for several hours at 80° or passed through two layers of filter paper. Mayer sought unsuccessfully to discover a causal microorganism by microscopic studies, poured plate cultures, and inoculations. Nevertheless, he concluded that the disease was caused by a bacterial organism that he had been unable to identify.

About the same time that Mayer was working on tobacco mosaic, Smith (1888, 1891) in the United States made a very thorough study of peach yellows. He could transmit the disease only by budding from diseased to healthy trees and obtaining graft union. He was unable to determine the cause of peach yellows, but thought it was similar to that of tobacco mosaic.

The next great step in the advancement of knowledge of virus diseases was passage of the infective entity of tobacco mosaic through a bacterium-proof filter. This was done by Dimitrii Ivanowski (1892), a Russian who worked at the Botanical Laboratory of the Academy of Sciences at St. Petersburg. Ivanowski confirmed Mayer's work on the transmission of tobacco mosaic and corrected Mayer's erroneous statement that the causal entity was lost when juice from diseased plants was passed through two layers of filter paper. He passed juice from diseased plants through a Chamberland filter and found that it retained its infectivity. Although this was the first recorded passage of the causal virus of a plant or animal disease through a bacterium-proof filter, Ivanowski did not fully appreciate the importance of his accomplishment. He still thought that the causal agent of tobacco mosaic was a bacterium which might in some way have passed through the filter, or might have produced a toxin that passed through.

The existence of viruses as causal agents of disease was generally accepted after the work of Beijerinck (1898), a brilliant Dutch botanist and microbiologist who brought the best bacteriological knowledge and methods of his time to the study of tobacco mosaic. Born in Amsterdam, Holland, in 1851, Martinus Willem Beijerinck was thoroughly trained in the physical and biological sciences at the University of Leyden, where he received his Ph.D. degree in 1877. Most of his work was done at the Technical School of Delft, where he founded the Microbiological Laboratory and became a great pioneer in soil microbiology. He confirmed Ivanowski's finding that the causal entity of tobacco mosaic would pass through a porcelain filter, and was unable to culture any organism from the filtrate. He proved that the infectious agent increased in the tobacco plant and that it could pass through a layer of agar. He concluded that the disease was not caused by a microbe or anything corpuscular, but by a "*contagium vivum fluidum*," which he also referred to as a virus.

Thus, at the beginning of the 20th century, the virological trend in plant pathology was definitely established, but its great development was yet to come.

## VII. PLANT PATHOLOGY IN THE 20TH CENTURY

The advent of the automobile and the airplane at the beginning of the 20th century intensified the influences of the steam age on plant pathology. As urbanization and transportation increased, crop plantings became more specialized, extensive, and concentrated; opportunities for spread of pathogens increased; and the problems of plant disease became more acute. With the recognition of these rapidly increasing problems, plant pathology, which hitherto had developed in departments of botany, became a vigorous young science in its own right. Chairs and departments of plant pathology were established; teaching, research, and extension services developed; phytopathological societies organized; channels of publication provided.

The major trends of work that had been established in the 19th century were continued and expanded. However, the impact of new knowledge and techniques brought about some major new lines of emphasis. Excepting only the development of the microscope and the discovery that microorganisms can incite disease, the discovery of the laws of heredity might well be considered the most important contribution yet made to plant pathology. Genetics affords the key to knowledge of variability, and variability is a basic aspect of most problems of plant disease. The rise of the science of genetics; the growth of botany, bacteriology, chemistry, and physics; the development of the electron microscope and of atomic physics; and the application of mathematics to biological

problems have opened up vast new opportunities for research in plant pathology.

It is possible to mention here only a few major lines of new or increased emphasis in phytopathological work in the 20th century:

Education and organization: introduction of plant pathology into universities and colleges; development of extension services for education and information of growers; expansion of governmental and private agencies dealing with plant diseases; establishment of phytopathological societies and journals.

Genetics of host plants in relation to the inheritance and the nature of disease resistance, the development of disease resistant plants, and the nature of host-parasite interactions.

Environment in relation to plant disease development; influences on host, parasite, and host-parasite interactions.

Genetics of pathogens in relation to the inheritance and the nature of pathogenicity and to the nature of host-parasite interactions.

Physiology of parasitism.

Virus diseases: nature and properties of plant-infecting viruses; interactions of viruses with plants and with animal vectors; disease development and control.

Improvement in materials and methods for chemical control of plant diseases.

Epidemiology and its relation to plant disease control: major factors that govern development of epidemics; rational orientation of control measures.

Diseases incited by nematodes; their occurrence, development, and control.

Regulation: inspection, quarantine, and certification.

#### REFERENCES <sup>a</sup>

- Adanson, M. 1763. "Familles des plantes," Vol. 1. Vincent, Paris. 189 pp.
- American Phytopathological Society. 1956. In commemoration: Isaac-Bénédict Prévost 1755-1819. *Phytopathology* **46**: 1.
- Arthur, J. C. 1885. Proof that bacteria are the direct cause of the disease in trees known as pear blight. *Botan. Gaz.* **10**: 343-345.
- Arthur, J. C. 1887a. Pear blight. *N. Y. State Agr. Expt. Sta. (Geneva, N. Y.) Ann. Rept.* **5**: 275-289.
- Arthur, J. C. 1887b. Important articles on pear blight. *N. Y. State Agr. Expt. Sta. (Geneva, N. Y.) Ann. Rept.* **5**: 300-315.
- Bacon, F. 1605. "The Advancement of Learning" (W. A. Wright, ed.), 5th ed., 1926. Oxford Univ. Press, London and New York. 376 pp.

<sup>a</sup> In cases in which the original publication was not available to the present writer, the date of the original is placed after the author's name and a later edition or a translation is cited, with its date.

- Beijerinck, M. W. 1898. "Concerning a Contagium Vivum Fluidum as Cause of the Spot Disease of Tobacco Leaves." Translated from the German by J. Johnson in *Phytopathol. Classics* No. 7, 1942. American Phytopathological Society, Cayuga Press, Ithaca, New York, pp. 33-52.
- Berkeley, M. J. 1845. Disease in potatoes. *Gardeners' Chronicle* 1845: 593.
- Berkeley, M. J. 1846. Observations, botanical and physiological, on the potato murrain. *J. Roy. Hort. Soc.* 1: 9-34. (Reprinted in *Phytopathol. Classics* No. 8, 1948.)
- Berkeley, M. J. 1847. (No title.) *Gardeners' Chronicle* 1847: 779.
- Berkeley, M. J. 1848. The potato disease. *Gardeners' Chronicle* 1848: 557.
- Berkeley, M. J. 1854-1857. Vegetable pathology. *Gardeners' Chronicle* 1854: 4 ff. (Reprinted in part in *Phytopathol. Classics* No. 8, 1948.)
- Berkeley, M. J. 1857. "Introduction to Cryptogamic Botany." Bailliere, London. 604 pp.
- Berkeley, M. J. 1860. "Outlines of British Fungology." Reeve, London. 442 pp.
- Biffen, R. H. 1905. Mendel's laws of inheritance and wheat breeding. *J. Agr. Sci.* 1: 4-48.
- Biffen, R. H. 1907. Studies in the inheritance of disease resistance. *J. Agr. Sci.* 2: 109-128.
- Biffen, R. H. 1912. Studies in the inheritance of disease resistance. II. *J. Agr. Sci.* 4: 421-429.
- Brefeld, O. 1875. Methoden zur Untersuchung der Pilze. *Landwirtsch. Jahrb.* 4: 151-175.
- Brefeld, O. 1881. Culturmethode zur Untersuchung der Pilze. In his "Botanische Untersuchungen über Schimmelpilze," Vol. 4. A. Felix, Leipzig. pp. 1-35.
- Brefeld, O. 1883. Die künstliche Cultur parasitischer Pilze. In his "Untersuchungen aus dem Gesamtgebiete der Mykologie," Vol. 5. A. Felix, Leipzig. pp. 1-28.
- Brefeld, O. 1888. Neue Untersuchungen über die Brandpilze und die Brandkrankheiten. II. *Nachr.-Klub Landwirte Berlin* No. 220, 1577-1584, No. 221, 1588-1594, No. 222, 1597-1602. (English translation by E. F. Smith in *J. Mycol.* 6, 1890-1891.)
- Brefeld, O. 1912. Die Brandpilze und die Brandkrankheiten. In his "Untersuchungen aus dem Gesamtgebiete der Mykologie," Vol. 15. H. Schöningh, Münster. 151 pp.
- Brefeld, O., and R. Falck. 1905. Die Blüteninfektion bei den Brandpilzen und die natürliche Verbreitung der Brandkrankheiten. In his "Untersuchungen aus dem Gesamtgebiete der Mykologie," Vol. 13. H. Schöningh, Münster. pp. 1-74.
- Buller, A. H. R. 1915. Micheli and the discovery of reproduction in fungi. *Trans. Roy. Soc. Can., IV* [3] 9: 1-25.
- Bulliard, P., and E. P. Ventenat. 1809-1812. "Histoire des champignons de la France." Leblanc, Paris. 2 Vols.
- Burrill, T. J. 1878. Report on botany and vegetable physiology. Pear-blight. *Trans. Illinois State Hort. Soc.* 11: 114-116.
- Burrill, T. J. 1881. Anthrax of fruit trees; or the so-called fire blight of pear, and twig blight of apple trees. *Proc. Am. Assoc. Advance. Sci.* 29: 583-597.
- Burrill, T. J. 1884. Pear blight and peach yellows. *Trans. Illinois State Hort. Soc.* 17: 46-49.
- Corda, A. C. I. 1837-1854. "Icones fungorum hucusque cognitorum," Vols. 1-4. J. G. Calve, Prague; Vols. 5-6 (J. B. Zobel, ed. of Vol. 6). F. Ehrlich, Prague.
- De Bary, A. 1853. "Untersuchungen über die Brandpilze und die durch sie verursachten Krankheiten der Pflanzen mit Rücksicht auf das Getreide und andere Nutzpflanzen." G. W. F. Müller, Berlin. 144 pp.



- De Bary, A. 1861. "Die gegenwärtig herrschende Kartoffelkrankheit, ihre Ursache und ihre Verhütung." A. Felix, Leipzig. 75 pp.
- De Bary, A. 1863. Recherches sur le développement de quelques champignons parasites. *Ann. sci. nat. Botan.* [4] **20**: 1-144.
- De Bary, A. 1866. "Morphologie und Physiologie der Pilze, Flechten und Myxomyceten." W. Engelmann, Leipzig. 316 pp.
- De Bary, A. 1866-1867. Neue Untersuchungen über Uredineen. *Monatsber. Königlich preuss. Akad. Wiss. Berlin* **1865**: 15-49; **1866**: 205-215.
- De Bary, A. 1876. Researches into the nature of the potato-fungus, *Phytophthora infestans*. *J. Botany, Brit. and For.* **14**: 105-126, 149-154.
- De Bary, A. 1881. Zur Kenntniss der Peronosporae. *Botan. Ztg.* **39**: 521-530, 537-544, 553-563, 569-578, 585-595, 601-609, 617-625.
- De Bary, A. 1884. "Vergleichende Morphologie und Biologie der Pilze, Mycetozoen und Bakterien." W. Engelmann, Leipzig. 558 pp. (English translation by H. E. F. Garnsey, revised by I. B. B. Balfour, 1887. Oxford Univ. Press, London and New York.)
- De Bary, A. 1886. Ueber einige Sclerotinien und Sclerotien Krankheiten. *Botan. Ztg.* **44**: 377-387, 393-404, 409-426, 433-441, 449-461, 465-474.
- De Bary, A. 1887. "Vorlesungen über Bakterien." W. Engelmann, Leipzig. 146 pp. (English translation by H. E. F. Garnsey, revised by I. B. B. Balfour, 1887. Oxford Univ. Press, London and New York.)
- della Porta, G. B. 1588. "Phytognomonica Jo. Baptistae Portae," 2nd ed., 1591. J. Wechelum & P. Fischerum, Francofurti. 552 pp.
- de Tournefort, J. P. 1705. Observations sur les maladies des plantes. *Mem. acad. roy. sci. Paris* **1705**: 332-345.
- Dobell, C. 1932. Antony van Leeuwenhoek and His "Little Animals." Staples Press, London. 435 pp.
- Dubos, R. J. 1950. "Louis Pasteur Free Lance of Science." Little, Brown, Boston. 418 pp.
- Ducleaux, E. 1896. "Pasteur. Histoire d' un esprit." Translated from the French by E. F. Smith and Florence Hedges, 1920. W. B. Saunders, Philadelphia. 363 pp.
- Fabricius, J. C. 1774. Forsøg til en Afhandling om Planternes Sygdomme. *Kgl. Norske Videnskab. Selskabs Forh., Skrifter* **5**: 431-492. (English translation by Mrs. M. Kølpin Ravn in *Phytopathol. Classics* **No. 1**, 1926.)
- Farrer, W. 1899. The making and improvement of wheats for Australian conditions. *Agr. Gaz. N. S. Wales* **9**: 131-168, 241-260.
- Fischer, A. 1897. "Vorlesungen über Bakterien." G. Fischer, Jena. 186 pp.
- Fischer, A. 1899. Die Bakterienkrankheiten der Pflanzen. *Centr. Bakteriolog. Parasitenk., Abt. II* **5**: 279-287.
- Fontana, F. 1767. "Observations on the Rust of Grain." Translated from the Italian by P. P. Pirone in *Phytopathol. Classics* **No. 2**, 1932. American Phytopathological Society, Hayworth Printing Co., Washington. 40 pp.
- Fries, E. M. 1821-1832. "Systema mycologicum, sistens fungorum ordines, genera et species, huc usque cognitae," Vols. 1, 3. Sumtibus E. Mauriti, Gryphiswaldiae. Vol. 2. Ex officina Berlingiana, Lundae.
- Greene, E. L. 1910. Landmarks of botanical history. *Smithsonian Inst. Publs. Misc. Collections* **54**: 13-329.
- Grew, N. 1682. "The Anatomy of Plants. With an Idea of a Philosophical History of Plants and Several Other Features," 2nd ed. W. Rawlins, London. 304 pp.
- Hales, S. 1727. "Vegetable Staticks." W. and J. Innys, London. 376 pp.
- Hartig, R. 1874. "Wichtige Krankheiten der Waldbäume." J. Springer, Berlin. 127 pp.



- Hartig, R. 1882. "Lehrbuch der Baumkrankheiten." J. Springer, Berlin. 198 pp. (English translation of 2nd edition by W. Somerville and H. M. Ward, 1894. Macmillan, London and New York.)
- Heuzé, G. 1852. *Procédé Grison. Rev. hort. (Paris)* [4] **1**: 168-170.
- Hooke, R. 1665. "Micrographia: or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses." J. Martyn and J. Allestry, London. 246 pp.
- Ivanowski, D. 1892. Ueber die Mosaikkrankheit der Tabakspflanze. *St. Pétersbourg Acad. Imp. Sci. Bull.* [3] **35** (also [4]3): 67-70. (English translation by J. Johnson in *Phytopathol. Classics No. 7*, 1942.)
- Jones, L. R., N. J. Giddings, and B. F. Lutman. 1912. Investigations of the potato fungus *Phytophthora infestans*. U. S. Dept. Agr. Bur. Plant Ind. Bull. **245**: 100 pp.
- Keitt, G. W. 1956. Isaac-Bénédict Prévost 1755-1819. *Phytopathology* **46**: 2-5.
- Koch, R. 1876. Die Aetiologie der Milzbrand-Krankheit, begründet auf die Entwicklungsgeschichte des *Bacillus anthracis*. *Beitr. Biol. Pflanz.* **2**: 277-310.
- Kühn, J. G. 1858. "Die Krankheiten der Kulturgewächse, ihre Ursachen und ihre Verhütung." G. Bosselmann, Berlin. 312 pp.
- Large, E. C. 1940. "The Advance of the Fungi." Henry Holt, New York. 488 pp.
- Léveillé, J. H. 1837. Recherches sur l'hymenium des champignons. *Ann. sci. nat. Bot.* [2] **8**: 321-338.
- Léveillé, J. H. 1846. Description des champignons de l'Herbier du Muséum de Paris. *Ann. sci. nat. Bot.* [3] **5**: 111-167, 249-304.
- Léveillé, J. H. 1851. Organization et disposition méthodique des espèces qui composent le genre *Erysiphé*. *Ann. sci. nat. Bot.* [3] **15**: 109-179.
- Liebig, J. (Not dated. 1853? a). Researches on the motion of the juices in the animal body; and the effect of evaporation in plants. Together with an account of the origin of the potato disease; with full and ingenious directions for the protection and entire prevention of the potato plant against all diseases. (W. Gregory, ed.). In "Liebig's Complete Works on Chemistry." T. B. Peterson, Philadelphia, pp. 3-47.
- Liebig, J. (Not dated. 1853? b). Chemistry in its application to agriculture and physiology (L. Playfair, ed.). Part II. Of the chemical processes of fermentation, decay and putrefaction. In "Liebig's Complete Works on Chemistry." T. B. Peterson, Philadelphia, pp. 87-130.
- Linnaeus, C. 1753. "Species plantarum." Impensis L. Salvii, Holmiae. 2 Vols.
- Lodeman, E. G. 1896. "The Spraying of Plants." Macmillan, New York. 399 pp.
- Malpighi, M. 1675-1679. "Anatome plantarum." J. Martyn, London. 2 Vols.
- Marès, H. H. 1856. Manual for the sulphuring of diseased vines and results. 3rd ed. in 1869. In Flagg, W. J. "Three Seasons in European Vineyards." Harper, New York. pp. 210-283.
- Mayer, A. 1886. Ueber die Mosaikkrankheit des Tabaks. *Landwirtsch. Vers.-Sta.* **32**: 451-467. (English translation by J. Johnson in *Phytopathol. Classics No. 7*, 1942.)
- Mendel, G. 1866. Versuche über Pflanzen-Hybriden. *Abhandl. Naturf. Ver. Brünn* **4**: 1-47.
- Meyen, F. J. F. 1841. "Pflanzen-Pathologie. Lehre von dem kranken Leben und Bilden der Pflanzen." Haude und Spenersche Buchhandlung, Berlin. 399 pp.
- Micheli, P. A. 1729. "Nova plantarum genera." Typis B. Paperinii, Florentiae. 234 pp.

- Millardet, P. A. 1878. Resistance au Phylloxera de quelques types sauvages de vignes américaines. *Compt. rend.* **87**: 739-740.
- Millardet, P. A. 1885a. Traitement du mildiou et du rot. *J. agr. prat.* **1885** (Vol. 2): 513-516. (English translation by F. J. Schneiderhan in *Phytopathol. Classics* **No. 3**, 1933.)
- Millardet, P. A. 1885b. Traitement du mildiou par le mélange de sulphate de cuivre et de chaux. *J. agr. prat.* **1885** (Vol. 2): 707-710. (English translation by F. J. Schneiderhan in *Phytopathol. Classics* **No. 3**, 1933.)
- Millardet, P. A. 1885c. Sur l'histoire du traitement du mildiou par le sulphate de cuivre. *J. agr. prat.* **1885** (Vol. 2): 801-805. (English translation by F. J. Schneiderhan in *Phytopathol. Classics* **No. 3**, 1933.)
- Millardet, P. A. 1891a. Nouvelles recherches sur la résistance et l'immunité Phylloxériques. Échelle de résistance. *J. agr. prat.* **1891** (Vol. 2): 839-843.
- Millardet, P. A. 1891b. Notice sur quelques porte-greffes franco-américaines résistant a la chlorose et au Phylloxera. *J. agr. prat.* **1891** (Vol. 2): 876-880.
- Millardet, P. A. 1894. Importance de l'hybridization pour la reconstitution des vignobles. *Compt. rend.* **119**: 1176-1180.
- Nees von Esenbeck, C. C. 1816-1817. "Das System der Pilze und Schwämme." Stahelschen Buchhandlung, Würzburg. 329 pp. (2 Vols. in 1.)
- Orton, W. A. 1900. The wilt disease of cotton and its control. *U. S. Dept. Agr. Div. Vegetable Physiol. and Pathol. Bull.* **27**: 16 pp.
- Orton, W. A. 1909. The development of farm crops resistant to disease. *U. S. Dept. Agr. Yearbook* **1908**: 453-464.
- Persoon, D. C. H. 1801. "Synopsis methodica fungorum." H. Dieterich, Gottingae. 706 pp.
- Plinius Secundus, C. "The Natural History of Pliny," Vol. 3 in 1855, and Vol. 4 in 1856, translated from the Latin by J. Bostock and H. T. Riley. Bohn, London. 536 pp. and 523 pp., respectively.
- Prévost, B. 1807. "Mémoire sur la cause immédiate de la carie ou charbon des blés, et de plusieurs autres maladies des plantes, et sur les préservatifs de la carie." Bernard, Paris. 80 pp. (English translation by G. W. Keitt in *Phytopathol. Classics* **No. 6**, 1939.)
- Prévost, P. 1820. "Notice de la vie et des écrits d'Isaac-Bénédict Prévost." Paschoud, Genève. 110 pp.
- Ré, F. 1807. Essay, theoretical and practical, on the diseases of plants. 2nd ed. in 1817, translated from the Italian by M. J. Berkeley in *Gardeners' Chronicle* **1849-1850**: 228 ff.
- Robertson, J. 1824. On the mildew and some other diseases incident to fruit trees. *Trans. Hort. Soc. London* **5**: 175-185. (In a letter to the Secretary, read Nov. 20, 1821.)
- Savastano, L. 1887. "Tuberculosis iperplasie e tumori dell' olivo I. II. Memoria." Flli. Ferrante, Napoli. 131 pp.
- Seward, A. C. 1931. "Plant Life Through the Ages." Macmillan, New York. 601 pp.
- Smith, E. F. 1888. Peach yellows: a preliminary report. *U. S. Dept. Agr. Div. Botany Bull.* **9**. 254 pp.
- Smith, E. F. 1891. Additional evidence on the communicability of peach yellows and peach rosette. *U. S. Dept. Agr. Div. Vegetable Pathol. Bull.* **1**: 65 pp.
- Smith, E. F. 1895. *Bacillus tracheiphilus* sp. nov., die Ursache des Verwelkens verschiedener Cucurbitaceen. *Centr. Bakteriöl. Parasitenk., Abt. II* **1**: 364-373.
- Smith, E. F. 1896. A bacterial disease of the tomato, eggplant, and Irish potato

- (*Bacillus solanacearum* n. sp.). U. S. Dept. Agr. Div. Vegetable Physiol. and Pathol. Bull. **12**, 25 pp.
- Smith, E. F. 1897. *Pseudomonas campestris* (Pammel). The cause of a brown rot in cruciferous plants. *Centr. Bakteriolog. Parasitenk., Abt. II* **3**: 284-291, 408-415, 478-486.
- Smith, E. F. 1899a. Are there bacterial diseases of plants? *Centr. Bakteriolog. Parasitenk., Abt. II* **5**: 271-278.
- Smith, E. F. 1899b. Dr. Alfred Fischer in the role of pathologist. *Centr. Bakteriolog. Parasitenk., Abt. II* **5**: 810-817.
- Smith, E. F. 1901. Entgegnung auf Alfred Fischer's "Antwort" in Betreff der Existenz von durch Bakterien verursachten Pflanzenkrankheiten. *Centr. Bakteriolog. Parasitenk., Abt. II* **7**: 88-100, 128-139, 190-199.
- Smith, E. F. 1905-1914. Bacteria in relation to plant diseases. *Carnegie Inst. Wash. Publ.* **1**, **2**, and **3**.
- Smith, E. F., N. A. Brown, and C. O. Townsend. 1911. Crown-gall of plants: its cause and remedy. *U. S. Dept. Agr. Bur. Plant Ind. Bull.* **213**: 215 pp.
- Smith, E. F., N. A. Brown, and L. McCulloch. 1912. The structure and development of crown gall. *U. S. Dept. Agr. Bur. Plant Ind. Bull.* **255**: 60 pp.
- Sorauer, P. 1874. "Handbuch der Pflanzenkrankheiten." Wiegandt, Hempel and Parey, Berlin. 406 pp. (Now in 6th ed., revised.)
- Sorauer, P. 1909. "Handbuch der Pflanzenkrankheiten," 3rd ed., Vol. 1. P. Parey, Berlin. 891 pp. (English translation by Frances Dorrance, 1922. Record Press, Wilkes-Barre, Pennsylvania.)
- Targioni-Tozzetti, G. 1767. True nature, causes and sad effects of the rust, the bunt, the smut, and other maladies of wheat, and of oats in the field. Translated from the Italian by L. R. Tehon in *Phytopathol. Classics* No. **9**, 1952. The American Phytopathological Society. Cayuga Press, Ithaca, New York. 139 pp.
- Tessier, M. l'Abbé. 1783. "Traité des maladies des grains." La Veuve Herissant, Paris. 349 pp.
- Theophrastus. Enquiry into plants and minor works on odours and weather signs with an English translation by Sir Arthur Hort. 1916. W. Heinemann, London and G. P. Putnam's Sons, New York. 2 Vols.
- Theophrastus. De causis plantarum, book one; text, critical apparatus, translation, and commentary [by] Robert E. Dengler. 1927. Westbrook Publ. Co., Philadelphia. 143 pp.
- Tillet, M. 1755. Dissertation on the cause of the corruption and smutting of the kernels of wheat in the head. Translated from the French by H. B. Humphrey in *Phytopathol. Classics* No. **5**, 1937. American Phytopathological Society, Ithaca, New York. 189 pp.
- Truffaut, fils. 1852. Procédé Bergman. *Rev. hort. (Paris)* [4] **1**: 170-172.
- Tulasne, L. R. 1854. Sur le dimorphisme des Urédinées. *Compt. rend.* **38**: 761-765.
- Tulasne, L. R., and C. Tulasne. 1847. Mémoire sur les Ustilaginées comparées aux Urédinées. *Ann. sci. nat.* [3] **7**: 12-127.
- Tulasne, L. R., and C. Tulasne. 1861-1865. "Selecta fungorum carpologia," 3 Vols. Imperial. typograph., Parisiis. (English translation by W. P. Grove, 1931. Oxford Univ. Press, London and New York.)
- Tyler, S. A., and E. S. Barghoorn. 1954. Occurrence of structurally preserved plants in pre-Cambrian rocks of the Canadian Shield. *Science* **119**: 606-608.
- Unger, F. 1833. "Die Exantheme der Pflanzen." C. Gerold, Vienna. 422 pp.
- Varro, M. T. On agriculture with an English translation by W. D. Cooper revised

- by H. B. Ash. 1936. W. Heinemann Ltd., London, Harvard Univ. Press, Cambridge, Massachusetts. pp. 160-529. (In a volume with Cato "On Agriculture.")
- von Sachs, J. 1875. "History of Botany." Translated from the German by H. E. F. Garnsey revised by I. B. Balfour. 1890. Oxford Univ. Press, London and New York. 568 pp.
- von Schweinitz, L. D. 1822. Synopsis fungorum Carolinae superioris. *Schriften Naturf. Ges. Leipzig*. **1**: 20-131.
- Wakker, J. H. 1883. Vorläufige Mittheilungen über Hyacinthenkrankheiten. *Botan. Centr.* **14**: 315-317.
- Wakker, J. H. 1889. Contributions à la pathologie végétale. I. La maladie du jaune, ou maladie nouvelle des jacinthes causée par le *Bacterium Hyacinthi*. *Arch. néerl. sci.* **23**: 1-25.
- Ward, H. M. 1888. A lily-disease. *Ann. Botany (London)* **2**: 319-376.
- Ward, H. M. 1902a. On the relations between host and parasite in the bromes and their brown rust, *Puccinia dispersa* (Erikss.). *Ann. Botany (London)* **16**: 233-315.
- Ward, H. M. 1902b. On the question of "predisposition" and "immunity" in plants. *Proc. Cambridge Phil. Soc.* **11** (Part 5): 307-328.
- Ward, H. M. 1903. Further observations on the brown rust of the bromes, *Puccinia dispersa* (Erikss.) and its adaptive parasitism. *Ann. Mycol.* **1**: 132-151.
- Weld, C. R. 1848. "A History of the Royal Society," Vol. 1. Parker, London. 527 pp.
- Whetzel, H. H. 1918. "An Outline of the History of Phytopathology." Saunders, Philadelphia. 130 pp.
- Woolman, H. M., and H. B. Humphrey. 1924. Summary of literature on bunt, or stinking smut, of wheat. *U. S. Dept. Agr. Dept. Bull.* **210**: 44 pp.





## CHAPTER 4

# How Sick Is the Plant?

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Since a plant has no blood pressure or pulse and can show no fever, the diagnostician sometimes has difficulty in knowing how sick is the plant. Since he has difficulty in measuring disease quantitatively in the single plant, he has difficulty in measuring it for the whole crop. Having gained ability to measure the sickness in the crop, there remains the complex problem of relating this to the amount of loss to the agriculturist or horticulturist. In this chapter, we shall deal with these interrelationships (see also Chester, 1955).

## I. SICKNESS AND LOSS

### *A. Sickness and Loss Are Different Concepts*

Sickness is the result of an abnormal physiological process, one that interferes with the normal functioning of the plant. The functions of the plant, from the biological viewpoint, are to survive, compete, grow, and reproduce its kind through flowering, fruiting, and disseminating its offspring. Whatever may interfere with these normal biological processes results in sickness, mechanical injury to, or violent destruction of the plant. Here our emphasis is on sickness, recognizing, however, that those concerned with the health of plants must embrace, in their thinking, plant damage resulting from the activities of man and the other

higher animals as well as that due to lightning, fire, flooding, hail, and other such violent destructiveness.

Man claims a proprietorship over plants, and is concerned with their sickness insofar as it interferes with his demands and expectations on the performance of the plant to serve his ends. When sickness in plants frustrates these demands and expectations, we have loss from the human viewpoint.

There is no regular relationship between sickness and loss. A population of plants may suffer devastating sickness, yet, if this does not interfere with man's desires, there is no loss from the human viewpoint. There may even be gain, as in the case of sickness in a noxious weed. Or the loss may be quite disproportionate to the injury suffered by the plant, as in the case of disfigurement of ornamental plants which does not seriously affect their life processes.

### *B. How Sickness and Loss Are Distinguished*

From the point of view of the individual plant, any kind or degree of sickness may be harmful, although plants, thanks to their capacity for replacing and restoring damaged organs, are able to tolerate greater destruction to their bodies than animals can. While much plant sickness results from the activities of parasites, parasitism in itself does not imply a corresponding degree of sickness. The conspicuous and widespread occurrence of powdery mildew on lilac in autumn, for example, is not associated with any important degree of sickness, since the attacked leaves have already accomplished their usefulness and will soon be shed. On the other hand, plants may suffer severe or fatal sickness from the effects of toxins released by microorganisms which are hardly to be classed as parasites.

From the point of view of plant population, sickness or death of the individual plant has little significance, provided the population maintains its competitive position in the plant world. In the long run, survival of the species or race is the important thing, regardless of how many individuals suffer or die along the line. Indeed, some loss of individuals is often beneficial to plant populations that are overcrowded owing to lavish reproduction. Forest trees offer many illustrations of this. The cottonwood tree, for example, has an enormous reproductive capacity. Customarily, newly exposed sandbars in major river bottoms become covered with scores of thousands of cottonwood seedlings per acre. All but a few hundred of these must die if the remaining trees are to attain normal maturity.

In other situations, sickness in certain individuals may endanger the

entire population. When, through chance or a high degree of susceptibility to an infectious disease, a few individuals contract the disease, they may serve as a source of inoculum which may spread to engulf the entire plant population over wide areas. This was the history of the chestnut blight in America, and it is constantly occurring in the rust diseases of cereals.

Looking at the matter from the point of view of the biotic complex, plant sickness may set off chain reactions that can profoundly affect the whole biosis. In brackish, muddy inlets of the North Atlantic seacoast of the United States, for example, the eelgrass, *Zostera marina*, is the dominant vegetation. Its presence provides substrate, food, and shelter for a myriad of marine animals and plants. When, a few years ago, the "wasting disease" of eelgrass wiped out this species over extensive areas, the whole microcosm of marine life underwent profound changes, directly through the loss of the plant and indirectly through changes in wave action, properties of the water, and altered navigation practices that resulted from loss of the eelgrass. We must note, too, the extensive results that follow the introduction of a disease agent on a single species of plant when, as with aster yellows, the pathogen exhibits its capacity for spreading to many other, unrelated species, quite altering the biotic complex. The special case of pathogens having alternate hosts deserves mention, in which introduced disease in one species of plant results in attack of another, quite unrelated species. In all such instances, sickness in one member of the biotic complex alters the complex and the welfare of its components. Obviously, this can be beneficial or harmful according to the component supplying the viewpoint.

Plant sickness from man's point of view is important insofar as it hinders or aids production of the commodity or result that he desires at the moment, and loss is a measure of the extent of that hindrance. Obviously, if potatoes are destroyed by blight or wheat by rust, man's purpose in growing these crops is frustrated. The loss is greater or less depending on the economic value attached to potatoes or grain that were sacrificed to the disease. But man's point of view is not single and simple. In these cases, what may have been serious loss to individual potato or grain growers may actually have been gain to their society if the diseases tended to reduce unmanageable surpluses of these commodities.

Man's point of view depends on who the man is. When the wilt disease began decimating populations of persimmon trees in southwestern United States this was regarded by some as causing serious loss of food for wildlife and of defense against soil erosion. Ranchers, on the other

hand, gained by the removal of a weed tree that is undesirably prevalent in pastures.

There are numerous instances in which plant sickness is useful and helpful to man. The ergot fungus on rye is propagated to provide medical supplies of the toxin in the ergot bodies. Pine trees have been inoculated with pathogenic fungi to increase the flow of the valuable resin. In the related field of animal pathology we recall the fostering in geese of the liver disease that yields the valuable *pâté de fois gras*. While it is earnestly hoped that man will never resort to the dissemination of agents of plant, animal, or human diseases as weapons of war, such use of disease would represent other instances in which sickness would be regarded as useful to man.

### *C. What Is a "Normal" Plant, Crop, or Plant Product?*

Plant sickness has been called an "abnormal" process. What, then is the norm from which the sick plant is deviated? Theoretically, the severity of a disease is the extent to which the diseased plant falls short of ideal development. Such an ideal probably never exists. In practice, "normal" commonly connotes "good average." To the U. S. Department of Agriculture the "normal yield" is that which occurs in good years over extended areas, and a crop exceeding this by 10% is regarded as a perfect, undamaged crop for the area (Valgren, 1922). In Germany, the "normal yield" is the theoretical yield for an entirely normal year, assuming average injury from pests, and in practice it corresponds to a 6- to 8-year average yield (Klemm, 1940). The latter is more realistic than the American standard, and avoids the absurdity of reports that particularly well favored crops have produced somewhat more than 100% of perfection, as well as the false implication that the utmost that can be achieved by a grower is an increase of a paltry 10% more than the local average in good years. In appraising loss we must compare sick plants with healthy ones, and, for practical purposes, the healthy or "normal" plant is similar in nature to the sick one and is growing under environmental influences, both physical and biotic, that are favorable and similar to those affecting the sick plant, except that the "normal" plant is free from the particular sickness in question.

### *D. How Does Plant Sickness Cause Losses?*

Most obvious are the direct losses resulting from reduction in the amount or quality of a useful product. These, however, set in motion a train of indirect losses, which, although important, are usually overlooked in reporting losses. Among the indirect losses may be decreased



purchasing power of the grower—as well as those dependent on this purchasing power—together with decreased activity, economic operation, and profits of the industries that are dependent on agriculture, such as grain elevators, mills, processing plants, railroads, banks, farm implement and chemical manufacturers, and others. The expense of replacing the missing produce by importation from regions outside those affected by crop disease, sometimes including the necessity of accepting less desirable substitute products, should also be included among indirect losses.

Actual losses include all direct and indirect losses. In addition, when a disease has been averted by the use of preventive measures—spraying, soil disinfestation, replanting, and others—the cost of these measures, plus the cost of the research that develops them and the educational programs that diffuse knowledge about them must be added to the sum of actual losses. In contrast, potential losses are those which would occur in the absence of preventive measures. Where economical disease control measures are available, the grower must choose the lesser of two evils, the actual loss from the cost of control if it is less than the potential loss in the absence of control.

We may also distinguish between recognized and hidden loss. The extent to which a “normal” crop falls short of its potential yield is hidden loss, and this may be very great. One form of hidden loss is the unnoticed restriction of growth of plants that are constantly subjected to city smoke and gases. Another is the subnormal nutritional value of some foods from crops that have suffered from environmental deficiencies, which is undetected in foods that are chosen solely on the basis of appearance. Yet another is seen in the wide variation in average, “normal,” yields per acre of a given crop growing in different areas.

Plant diseases may be classified according to their manner of causing losses, for example into: (a) diseases that seriously affect the normal life of plants, frequently killing them, as in the wilt diseases and damping-off; (b) diseases that destroy the commercial parts of the plant, as the smuts of small grains or the fruit rots; (c) diseases that destroy the reproductive organs (overlapping “b”); (d) diseases that stunt or retard the growth, or weaken the plant, without killing it, as is true of many virus diseases; (e) diseases that indirectly injure the commercial product by attacking other plant organs, as the foliage diseases of root, fruit, nut, and seed crops; (f) diseases that confer poisonous or other undesired properties on the product, as ergot of rye or scab of barley; (g) diseases that attack harvested products in storage, commerce, or home; (h) diseases that injure the attractiveness or aesthetic qualities of the product, such as peach freckle, apple fly speck, and blemishes of ornamental

plants; (i) mixed and intermediate types, with combined features of two or more of the foregoing classes.

Alternatively, diseases may be classified according to the degree of loss they cause, ranging from (a) those that practically eliminate the crop unless rigidly controlled, to (b) those that are quite destructive but sporadic, (c) those which are only occasionally and locally important, (d) those that are widespread and common but without important yield-depressing effects, and (e) those which ordinarily have little or no economic significance.

Other factors being equal, a disease that causes wide fluctuations in crop yield from one season to another causes more economic harm than another disease which causes equal cumulative reduction in yield but in about the same amount each season. Certain diseases are most severe in crops that are highly vigorous, as is characteristic of many diseases caused by rust fungi and other obligate parasites, along with downy mildew fungi and bacteria. Other diseases are most damaging in plants of low vitality, such as is often true of the wilts, root rots, cankers, and wood decays. Diseases of the former type tend to reduce fluctuations in crop yields while those of the latter type increase these fluctuations. These relationships may be expressed as a coefficient of correlation,  $r$ , between disease loss and potential yield in the absence of disease. If  $r$  is negative, the disease increases annual yield variation, while if  $r$  is positive the disease is associated with reduced yield fluctuation. (Hartley and Rathbun-Gravatt, 1937). Thus, for cotton wilt,  $r = -0.36$ , increasing variability, while with potato late blight  $r = +0.82$ . In the latter case, complete control of the disease would increase the yield variability, and late blight may be regarded as a stabilizing factor in potato production under conditions of the observations. This apparent beneficial effect of diseases with positive  $r$  values does not apply when the diseases attack with epidemic force, causing heavy losses over extensive areas, i.e., when the disease is more important than weather fluctuations or other factors contributing to crop vigor.

With diseases that are transmitted in the reproductive parts of plants, the amount of disease, and consequent loss, is cumulative, increasing from one plant generation to the next. This is characteristic of the tuber-borne diseases of potatoes, in which the long recognized "running out" of potato varieties is now known to result from the progressively increasing content of one or several viruses in the tubers, vegetative generation after generation. With soil-borne diseases, such as the root rots, a comparable cumulative loss effect is observed.

In perennial plants, sickness is often cumulative. Beyond the yield loss in the current season of attack, for example by a defoliating disease

of trees, the plant may be weaker, with less reserves, as it enters the following season of growth, and this weakness may increase cumulatively from year to year until death ensues. An example of this is anthracnose on white oak.

Plant diseases may reduce yields without affecting the market quality of the harvested crop (loose smut of wheat except when grown for seed), or may reduce quality without affecting yield (fruit blemishes), or, most commonly, diseases reduce both yield and quality. Usually, loss statistics include only quantitative loss, although the loss in quality may have even greater importance. Variations in market quality have complex effects on the marketing, and, in general, the effects of lowered quality are harmful to all concerned.

Among the forms of indirect loss caused by plant diseases, is their effect on the use and value of land. In many cases the disease hazard is as important a characteristic of land as its fertility, water supply, and topography. When a normally profitable crop cannot be profitably grown in certain areas because of its propensity to disease, and when other, less desirable crops must be grown, the land loses some of its utility, attractiveness, and value, as is illustrated by land that is infested with organisms which cause wilt disease, pathogenic nematodes, or the Texas root rot fungus.

Now and then, in the history of agriculture, a new disease of devastating potency assails a crop, drastically curtailing its production. When the disease first appears, its inroads lead to scarcity of the crop, usually attended by higher prices. This stimulates the use of substitute products, demand for the scarce commodity falls, and eventually the loss in volume is compounded by a loss in price as well. This secular effect of such devastating diseases is well illustrated by the *Endothia* blight of the American chestnut.

#### E. How Much Sickness Is Important?

The importance of plant sickness, to man, is a function of: (1) its destructiveness, (2) its timing and frequency, and (3) the value of the crop and its significance in the economy of the nation, the community, and the individual farmer. The first of these, destructiveness, which is often the only factor considered in loss statistics, is the product of the degree and nature of damage to individual plants multiplied by the frequency of injured plants in the population.

Of two diseases of equal destructiveness, ordinarily that which appears early in the growing season is more important than the one appearing later. Interference with the physiology of the plant, such as through the loss of photosynthetic tissue, is usually less harmful with

advancing age of the plant. The crops from early harvests frequently command the highest prices, which would aggravate the monetary loss from early season destructiveness of a plant disease.

The importance of a disease also rises with the frequency with which a crop is subjected to it. An apparent exception to this principle is the situation in which a disease is practically always present and causes about the same amount of loss every season. Diseases that occasionally break out with explosive force are less dangerous, in one sense, than those diseases that are always present to about the same extent. We are prone to consider these constant diseases like rats, taxes, soil erosion, highway fatalities, and the common cold, as "normal" or inevitable. We tolerate them and often forget or never realize that their constancy and our acceptance of it may constitute their most dangerous feature. Examples are wood decay in the forest, spoilage of fruits and vegetables in marketing and in the home, leaf spots of barley, and defoliation diseases of pasture plants. Our susceptibility to influence by the spectacular or infrequent leads us to overestimate the losses from such hazards, while we underestimate the destructiveness of the common, constant ones.

Other factors being equal, the importance of a disease increases with the value of the crop. Sickness in feed crops, such as sorghum, barley, and pasture plants, is regarded as less important than a comparable degree of sickness in more valuable food and industrial crops. When any crop assumes strategic importance, as in wartime, or when it is needed but in short supply, the importance of sickness rises proportionately. With ornamental plants in commerce and about homes, or with shade trees, a high value is attached to their appearance as well as their health, and correspondingly great importance is attached to disfiguring disease in them. A very striking example of this is the Dutch elm disease.

The importance of a plant disease declines with the ease and economy with which it can be controlled. From this viewpoint, such diseases as potato late blight and the cereal smuts are less important today than formerly, while those virus diseases that are largely uncontrollable have become relatively more important. The importance of a disease, when controlled, increases with increasing cost of the control measures.

#### *F. What Do Losses from Plant Diseases Signify?*

Statistics on the importance of plant diseases can be very misleading. When we consider the effect of plant disease purely from the point of view of total national production and national prices, at first sight it appears that in a free economy, diseases are often beneficial to the farmer, since reduced production is usually more than offset by increased



prices, a large crop actually being worth less money than a smaller one. This is brought out by statistical demand curves that relate production to price.

In a comprehensive study of demand made by H. S. Schultz (1938), it was found that of 10 major crops all had inelastic demand curves. With corn, a 0.5% decrease in production led to a 1% increase in price. With cotton, a 1% increase in supply depressed the price by 1.4%. A 1% increase in supply of wheat reduced the price by 2%. With sugar, hay, potatoes, oats, and barley, 1% increase in production resulted in price decreases of 2.5–3.3%, 2.3%, 3.3%, 1.67%, and 2.56% respectively, a bigger crop of any of these bringing the farmer a smaller return on the national average.

Are we to conclude that agricultural science, or specifically plant pathology, is harmful insofar as it increases production, thereby reducing farm income? If we do, we must sanction farm programs that reduce the productive power of farmers, we must close our eyes to the millions of nonagricultural consumers to whom decreased production means higher prices that buy poorer quality, and we must close our hearts to the many more millions of people throughout the world to whom anything short of maximum production means malnutrition or death by slow starvation.

We have momentarily assumed, as a general principle, that because of inelasticity of statistical demand curves of some leading farm crops, the farmer gains when production is curtailed. Were the losses from plant diseases equally prorated among all farmers, we could disregard individual differences, but they are not. Great variations in yields and losses may occur on adjacent farms in the same season. For those who are not close to the land there may be comfort in the statistic that in 1954 the average wheat acre in the United States produced 18.1 bushels of wheat worth \$2.13 per bushel. How little this means in human values to the farmer who harvested 5 bushels per acre while his neighbor harvested 30! Some diseases, such as root rot, are like that. Average national losses from disease, serious though they are, have but a small fraction of the social significance of the multitudes of individual catastrophes that are overlooked in the national or state averages.

The economic history of plant pathology, although never yet fully assembled, and existing principally in scattered items, is a tragic chronology of disaster after disaster which scourged the land, wiping out the livelihood of countless families, communities, and whole agricultural sections, destroying enterprises on which hopeful farmers had staked their lives and all their resources. There is a formidable list of agricul-



tural projects that have failed as a result of plant diseases. Some of these have virtually eliminated industries on which extensive areas depended. Examples are the collapse of the Louisiana sugar cane industry when it was successively crippled by red rot, root rot, and mosaic; the fate of the sugar beet industry in the intermountain area, throttled by the curly top disease; and the elimination, by rust, of coffee growing in Ceylon in the 1880's, and the culture of *Coffea arabica* in Java. Calamities such as these eliminated the livelihood of large populations, closed mills and factories, transformed prosperous communities into ghost towns.

Less spectacular, although no less ruinous to many individual farmers, and those dependent on farming, have been the many other instances in which disease has struck locally or on scattered farms, eliminating the culture of once profitable crops, forcing countless individual farm families off the land or into other, less attractive agricultural pursuits. The many plant diseases that have acted in this fashion include banana wilt, flax wilt and rust, sweet potato surface rot, wheat stem and leaf rusts, potato and tomato late blight, bacterial wilt of alfalfa, rust of asparagus, *Fusarium* wilts of watermelon and cotton, Granville wilt of tobacco, diseases of celery, downy mildew of grapes in France, and gooseberry powdery mildew in much of Europe. In these cases, the destruction of crop culture has not always been permanent; sooner or later plant scientists have found means of controlling many of these diseases or have developed profitable substitute crops. Yet, during the period of reorganization of farming, untold suffering has been undergone by the stricken farm populations. However closely we may attempt to arrive at estimates of the cost of plant diseases, our figures will always fall short of the true cost by a broad margin of intangible suffering that cannot be measured in dollars.

The consumer has an even greater stake in crop loss prevention than does the farmer. Whatever the losses in agriculture, it is the consumer who must absorb them in higher prices, lower quality, and taxes to permit the farmer to operate despite agricultural hazards. The consumer's stake is all the greater because the unit value of produce, when it reaches the consumer, is much higher than at the farm. "The consumer's apple is the producer's apple plus the cost of picking, packing, shipping, storage, and handling, as well as sales costs and profits" (Stevens, 1933). A farm loss, measured in pennies per bushel, becomes a consumer's loss measured in pennies per pound or dollars per bushel.

The farmers' loss, which may be offset to some extent by higher prices or subsidies, involves only the hazards that exist up to harvest

time. The consumer's loss includes these, plus all the forms of loss that occur between harvest and the dinner table, and these post-harvest losses may be relatively greater than losses on the farm. It is not uncommon for 25 to 50% of perishable produce to be lost between farm and home.

Accompanying volume losses at all stages in production and marketing are the quality losses in produce that finally reaches the ultimate consumer, seen, for example, in scabby potatoes from which a thick, wasteful paring must be removed, blemished fruit that is unappetizing and is subject to rapid decay in the home, leafy vegetables from which a wastefully large number of leaves must be removed before reaching the uninjured core, and construction timber with incipient stages of decay that inevitably mean costly, early replacement.

## II. HOW IS SICKNESS IN PLANTS RECOGNIZED AND DIAGNOSED?

### *A. Symptoms as Indicators of Affected Life Processes*

Sickness results from abnormal physiological processes in plants. Physiological abnormalities produce symptoms of disease. Sometimes these are very obvious, as in yellowing, wilting, or death of tissues; in other cases they may be very obscure, recognizable only by careful measurements, as with moderately retarded growth that appears normal, or with reduced reproductive capacity or seed viability. Symptoms are not the disease, although one might be led to think so from the common names of most diseases, such as aster yellows, cotton wilt, or barley stripe. Symptoms are only evidences of disease, recognizable responses of the plant to physiological disturbances. They are often accompanied by signs of disease, a term that is applied to evidences of the presence of a disease inducing agent, such as the fruiting bodies of pathogenic fungi, or bacterial ooze.

In phytopathology, as in veterinary and human medicine, it is customary to diagnose the disease from its symptoms and signs, to formulate a prognosis of the probable outcome of the sickness, and to endeavor to control it. In each of these sciences, control measures are properly directed at the underlying physiological abnormality or at the agent that causes it, not at alleviation of the symptoms themselves, aspirin and tranquilizing drugs notwithstanding.

### *B. The Individual Plant*

Diagnosis begins with the study of individual affected plants. A highly detailed science of symptomatology in plants has been developed, particularly under the aegis of the late Professor Whetzel of Cornell

University. It is not the purpose to elaborate here on this since it is fully discussed in most textbooks of plant pathology and since the relations of symptoms to abnormal physiological processes are considered in Part II of this volume. It need only be mentioned that sickness in plants is expressed by restricted development, excessive development, or death of the plant tissues, each of these reactions taking many forms.

The diagnostician attempts to understand the nature and degree of plant sickness from symptoms and signs. At best, this may be quite difficult and often the diagnostician is handicapped by being asked to diagnose from a few carelessly selected and handled specimens that are atypical and in poor condition for examination, perhaps even exhibiting the effects of a combination of unfavorable influences.

There is no good substitute for diagnosis of plant sickness in the presence of the growing plants. Here the diagnostician can get the "feel" of the whole problem, his judgment can be aided by appreciation of the extent and typical severity of the sickness, the environmental influences, the cultural practices, and the views of the growers.

The diagnostician must understand the physiology of the plant, in health and in sickness, in order to interpret symptoms. The writer, in abysmal ignorance of the practical aspects of wheat culture in Oklahoma, successfully forecast losses from the disastrous 1938 wheat leaf rust epidemic; these, in his mind, were inevitable in view of the dependence of yield on photosynthesis and the observable effect of the disease, long before harvest, in curtailing photosynthesis in the wheat plants over vast areas (Chester, 1939).

### *C. The Population*

Mycology was the forerunner of plant pathology. The weaning process has been difficult. Even today, there are plant pathologists whose conclusions are based on what they see through a hand lens, to the exclusion of field glasses. They suffer from a common ailment in the profession—mycological myopia. They cannot see the forest for the trees.

But plant pathology—with no disparagement to its basic scientific aspects—emphasizes the art of dealing with diseased plants. From this viewpoint, the individual sick plant means nothing—the sick population is paramount.

The diagnostician must know what percentage of the population is affected and to what average degree. The yellowness of a leaf should have less importance to him than the yellowness of the landscape. He requires the statistical approach. He must relate this, on the one hand, to the physiology of the sick plant or even the sick cell, and on the other

hand, to productiveness of the population as a whole. This may create a serious problem for the diagnostician if he has no normal population for comparison with the sick one. Ranking high among the symptoms of plant sickness—yet often overlooked—is the gross yield of the crop and its market grade.

#### D. *Chronic versus Acute Sickness*

As examples of chronic sickness in man we have the short stature, eye defects, and limited life span of certain peoples, also the high incidence of nonfatal respiratory ailments of residents in smoky cities. Plants, too, suffer from chronic ailments that restrict their development without producing more obvious symptoms of disease. Chronic sickness in plants is revealed in the differences in average acre yields of crops from one territory to another and in the differences in width between growth rings in the same species of tree growing in different localities. Often the statistical approach is the only one that will reveal that certain populations are failing to reach "normal" or potential productiveness, owing to pathogenic factors, whether environmental or biotic in nature. Chronic sickness is commonly overlooked, yet may often be more harmful to the population than obvious, acute, spectacular sickness.

#### E. *The Tempo of the Advancing Process of Disease Development*

The outcome of a horse race is determined not so much by the position of the horses at any given moment as by the speed at which they are running. So, too, with plant sickness; a single inspection may give very little indication of the dynamics of disease development. It is like inspecting a single frame of a moving picture. Just as an experienced seaman can determine the course and speed of a distant ship by signs that are meaningless to the landlubber, so the phytopathologist can learn to recognize the evidences that a plant disease is accelerating, static, or decreasing in intensity. It is important to give attention to the dynamics or tempo of disease development, since this increases our ability to foresee future loss, sometimes early enough to permit the intervention of loss-preventive measures.

Barratt and Richards (1944) studied the disease tempo of the target spot disease of tomato caused by *Alternaria solani*. Reading the disease at intervals as the season advanced they showed that the probability of disease is linearly related to time. Thus was formed a new technique for appraising the amount of sickness. The curves provide two very useful parameters, slope and half-life. The slope is a characteristic of the



population of plants, and the half-life is the time for 50% of the healthy tissue to be lost. The two parameters are very valuable in appraising fungicides, varietal susceptibility, environmental effects, and the like.

Large (1945) independently discovered and applied these two parameters extensively (Large 1952, 1958). It seems astonishing that so few research workers make use of them. Van der Plank is one of those who has (see Chapter 7 of Volume III).

A part of the problem of the march of disease is that available green tissue diminishes as the disease advances. Gregory (1948) gives a fascinating discussion of the mathematics of this phenomenon.

### III. WHAT IS THE VALUE OF KNOWING THE DEGREE OF SICKNESS IN PLANTS?

Accurate, measured data are fundamental to the understanding of any science. This is more difficult in biology than in the physical sciences, but nonetheless necessary. The measurements that can be made of the intensity, extent, and destructiveness of plant sickness find a wide variety of uses. For example, they enable us (a) to judge the relative importance of different kinds of disease, (b) to direct activities of research and extension to those that are most harmful to the economy, (c) to decide which of two control measures to use, one that is costly but efficient, the other less expensive and less efficacious; and (d) to obtain quantitative data in research to compare susceptibility of varieties, fungicides, environment, and the like. That is to say that if we are to advance the science of plant pathology and the art of treating disease, we must be able to express the amount of sickness in quantitative terms.

For all these reasons, governments have set up agencies for gathering and reporting plant disease information. The Ninth International Conference on the subject was held in Moscow in 1958 (Anon., 1958). The Food and Agriculture Organization of the United Nations publishes The Plant Protection Bulletin from Rome. Wood (1953) has shown the importance of plant diseases in the economy of that nation. Padwick (1956) has compiled a list of plant diseases in the British colonies. These are examples of the type of work carried on by all nations.

Forest disease appraisal illustrates how knowledge of the amount of advancing disease in a crop can help in determining present and potential sales value of the crop: using well tested techniques, the timber cruiser can determine the amount of wood decay, relate this to annual increase in the apparent and real volume of wood present, and thus determine the value of the forest, at present, and projected into future



years. This permits intelligent financial operations in managing and marketing the crop of timber. Other than in forest pathology, much remains to be done in securing and using disease loss data in relation to buying and selling farm property, farm taxation, farm mortgages, loans, credits, and crop insurance against disease losses.

An important service is rendered to agriculture by the periodic crop news and yield forecasts issued by agricultural economists. There are numerous cases where plant diseases, acting over a wide area, produce important downward revisions of yield estimates by harvest time. The crop reporter needs to know the relative yield-depressing effects of the different diseases, and for each important disease, insofar as possible, he needs to know that a given intensity of disease at a given stage in crop development is regularly followed by a given percentage reduction in crop yield at harvest time. Such information can contribute materially toward the accuracy and timeliness of yield forecasts, with their benefits in more orderly marketing of the crops.

Knowing the effect of given intensities of disease on yields, it becomes possible to interpret the role of plant disease in the production totals, to determine the extent to which new disease control measures may influence future production, and to gain some conception of the levels of production that are attainable with increased disease control. The analysis of commodity price variations and the forecasting of prices for crops will frequently be improved by definite knowledge of the effect of a given disease situation on quality as well as quantity of harvested crops.

Harmful effects of plant disease often occur after harvest, during the marketing of produce. If we had a comprehensive and reasonably accurate basis of data for evaluating market losses in their true light, it would become recognized that such losses are not inevitable, and efforts at their prevention would be justified and facilitated, with benefits to both marketer and consumer.

Timely and accurate knowledge of crop losses is essential in making economical and profitable disposition of harvested crops, in dispatching suitable numbers of railroad cars or trucks to harvest points, in planning canning and packing operations, and in managing crop storages. The marketing of equipment and supplies for controlling plant diseases is particularly dependent on factual information concerning the losses they cause, which determines the control expenditures that may be warranted.

There is a long list of agricultural enterprises that have failed because of the onslaught of plant sickness. In most such cases, the hazard could have been foreseen had there been appreciation of the destructiveness

of the diseases in question and knowledge of their occurrence or adaptation in the areas of proposed projects. An adequate basis for predicting the influence of plant sickness on contemplated agricultural ventures comprises: measurement of the damage which diseases, at given intensities, are capable of producing; determination of past extensions of disease areas and of their present areas by survey methods; study of the ecology of diseases to determine the likelihood that a given disease could prosper in a new location and environment; and a summarizing of this information in disease hazard maps to be used in agricultural planning, in the same manner and with the same advantages as land-use maps or soil-survey maps.

Accurate knowledge of the capacity of plant disease to cause losses is basic in determining the limits of safe exchange of agricultural and horticultural products, and in guiding disease-regulatory activities. The necessity for, and values of, disease control by embargo or regulation are functions of the amount of loss the disease is capable of producing. The threshold loss amount, above which regulation is justified, and below which the cost and consequences of regulation would not be warranted, should be the deciding factor in weighing the desirability of regulatory measures. The capacity of a disease to cause loss cannot be guessed at; it must be measured.

The prosperity of plant pathology as a science depends importantly on the financial support which it receives. This support, in turn, is contingent to a major extent on the ability of plant pathologists to demonstrate the economic value of their work. The latter, finally must rest on the accumulation of reliable data showing in reasonably accurate terms the amounts of loss caused by the various diseases and, consequently, the gain from disease control that has been attained or is in prospect. From this point of view, the securing of these data, the measurement of plant disease losses on a broad and comprehensive scale, is not just another optional facet of pathological studies; it is vital to the prosperous future of the science.

#### IV. WHY MUST THE DEGREE OF SICKNESS IN PLANTS BE MEASURED?

##### *A. Action against Plant Diseases Must Be Based on Accurate Information*

Ours is a military campaign against agents that destroy our plants. We cannot wage this campaign successfully without knowing the measure of the enemy's ability to destroy. To determine this (and our own vulnerability) is a function of our military intelligence service, without which we are unable to marshal our defensive forces when and where

they are most needed. Our intelligence service must furnish us with measured, exact information on the enemy's capacity for destruction. Guesses will **not** do.

### *B. Progress in Our Science Requires This Exact Information*

Ours is a science that can flourish only if it is based, in all its aspects, upon accurately measured data, without which it is not a mature science. The history of medicine through the past century shows clearly how its progress has depended on accurate measurements of structures and functions of the healthy and the sick, resulting in more precise diagnosis, more effective treatments, and, from the statistical study of sickness and morbidity in populations, a more rational concentration of medical efforts on those diseases that are truly most harmful to man. Phytopathology can profit from this example.

### *C. Our Present Data on the Degree of Sickness and Loss in Plants Are Very Fragmentary*

Plant disease surveys have never been highly organized and strongly supported, with the result that existing data on plant disease occurrences, intensities, and resulting losses are incomplete and nonrepresentative. There has been a tendency to report only extreme cases of disease outbreak, from which destructiveness averages cannot be derived. Many reports are of disease occurrences only, without information on their severity. Many others indicate severity by such general terms as "worse than usual," "very injurious," or "unusually prevalent," which convey little meaning to the worker who is unfamiliar with the average situation in the area concerned, and none to the analyst who is attempting to place disease severity on a numerical basis. It is often impossible to determine from the reports whether disease outbreaks are general over a wide area or localized on a few farms. The data from some agricultural areas are much less complete than those from areas that are better staffed. Owing to the personal research interests of individual reporters, to the spectacular character of some diseases contrasted with the more subtle destructiveness of others, and to other factors, we find some crops and diseases much better documented than others.

### *D. Our Present Data on the Degree of Sickness and Loss in Plants Are Very Inaccurate*

Lacking standard methods for scaling disease intensity, and with little experimental basis for determining the losses caused by plant diseases, our estimates of these losses have often been in serious error,

as has been seen when estimates have been compared with actual measurements.

Horsfall (1930) mentions workers who believed that no damage was caused by powdery mildew of clover, but his measurements showed that the disease reduced the crop by 25 to 33%. Wheat leaf rust was considered negligible or even beneficial to wheat until Mains (1930), Johnston (1931), Caldwell and Compton (1939), and others demonstrated experimentally that the disease reduces the crop by 35% if it destroys the leaves when the plant is in the blossoming stage, as frequently happens. Many other examples of gross inaccuracy in our conception of disease losses could be cited.

There are many reasons for such inaccurate estimates. There may be failure to ascribe loss to its actual cause, as when unfavorable weather is blamed for disease losses, in cases of damping-off and root rots, for example. There is often failure to appreciate the destructiveness of factors that are relatively constant from year to year and not spectacular nor widely publicized, as in the cases of clover mildew and wheat leaf rust mentioned above. If a disease is invariably present in a crop, the amount of loss which it causes may be underestimated or overlooked because of lack of contrast with disease-free plants. This was the case with potato latent mosaic, which was present in practically every potato plant grown in America, until its damage was measured and found to average 13% loss of the crop.

Often there is a lack of data on healthy crops to temper reports of epidemics, resulting in a distorted impression of the importance of diseases. Certain diseases tend to be most active in seasons of high potential crop yield, which obscures the actual losses sustained. This is particularly true of diseases that are favored by abundant rainfall, in dry regions where the benefits of the rainfall obscure their harmful effects. This applies to many of the rust and downy mildew diseases. If, as sometimes happens, there is a positive correlation between the yield-depressing effect of a disease and the yield-elevating effect of freedom from another disease or hazard, the two effects may cancel one another, or if the second effect be greater there may actually be a net yield increase associated with the disease. This has been reported of diseases which shorten the life cycle of plants, permitting them to mature their fruits early enough to escape frost damage.

An estimate of loss due to a plant disease must include all of the losses sustained, both in the growing plant and in the shipment, storage, and marketing of its products. There are diseases from which field loss is greater than is indicated by condition of the harvested crop, such as bunt of wheat, in which case examination of properly cleaned wheat



grain would suggest a much smaller amount of disease in the field than was actually present. Conversely a disease that is considered negligible in the field may cause serious post-harvest losses, as is frequently true of tomato anthracnose.

Other inaccuracies in disease loss appraisal result from subjective errors of judgment owing to inadequate or biased training and experience, from nonrepresentative sampling, from using an inappropriate method of appraisal or from duplicating and summing loss estimates at different stages in the marketing of a crop.

Basic to all these pitfalls is lack of an experimental basis for estimating disease losses. Many examples might be cited showing clearly that plant pathologists cannot trust their eyes or even their experienced judgment where there is no experimental basis for knowing the amount of loss associated with a given intensity of disease.

### *E. The Harmful Effects of These Inaccuracies*

In Section III, above, was discussed the value of accurate information on the degree of sickness in plants and the resulting economic losses in connection with research and educational work and with many aspects of agricultural economics. It is patent that if this information is inaccurate it may not only fail to support each of these activities but may even be harmful to them. It is quite probable that the discipline of phytopathology, with all of its yet unrealized contributions to our economy and science, has seriously suffered, in its development, from lack of adequate understanding of the economic consequences of plant sickness.

## V. WHAT ARE THE REQUIREMENTS IN MEASURING SICKNESS IN PLANTS?

### *A. The Objective*

The objective of plant disease loss appraisal is threefold: to determine the amount of disease, which is the product of its prevalence and its intensity in individual plants; to translate the amount of disease into loss, expressed as percentage of potential, disease-free crop, or in production units, considering both quantity and quality of the crop; and to interpret the effects of this loss on the economy. It is recognized that the last of these, the interpretation of the effects of loss, is a problem for economists and sociologists and lies outside the domain of our experimental science. Nevertheless, it is a very necessary part of the loss problem. Unequal progress has been made toward these objectives. Considerable attention has been given to measuring the amount of plant sickness,



much less to measuring the loss caused by it, and least of all to the socioeconomic interpretation of this loss.

### B. *The Methods*

The methods for measuring sickness in plants should meet certain requirements. They should be comprehensive, ultimately embracing all major diseases of all major crops, otherwise the assembled data will have only limited value for the important purpose of comparing loss hazards in order to determine the wisest course in research, educational and action programs.

Disease appraisal methods should have a practical degree of accuracy. Between the extremes of gross error on the one hand, and minutely precise measurements on the other, there is a suitable range in which disease and loss estimates are sufficiently accurate to be useful and reliable within moderate limits, but without reaching an uneconomical degree of precision or one that is unattainable in practice. The width of the permissible range of error of estimates depends on several factors, including the experimental basis for estimation, variability of loss from given diseases, purposes of the estimates, and practical considerations.

Disease appraisal methods should be comparable from one worker, location, or season to another. First, there are required comparable or uniform practices in appraising disease intensity. Some progress has been made in this direction, for example through the use of a standard scale for estimating cereal rust intensities. Second, there is need for standard, experimentally determined conversion factors, formulas, or regressions to translate disease intensity into disease loss. Some of these have already been derived; many more others await development.

Disease appraisal methods should be objective. They should be so devised that their use will not be influenced by bias or point of view of the observer. The true scientific observer recognizes bias as an ever-present danger in his work, and will welcome objective criteria for disease intensity and loss appraisal.

The methods should embrace all components of disease loss, including quantity and quality of crop yield and the indirect economic effects of disease, from planting to final disposal of the crop. Some cases are complex, with the loss difficult to analyze. This is a challenge, since understanding of the loss in these cases may justify new efforts and new approaches to the control of those disease problems where the loss is serious, although complex and obscure. Loss in quality of the crop, although sometimes difficult to appraise, may have greater significance than loss in yield. This is illustrated by tobacco mosaic, where infestation of the tobacco crop one month after transplanting reduced the acre

yield by 25%, but so lowered the quality that the price dropped by 40%, reducing the acre value by 54.5% (McMurtrey, 1928, 1929). Nursery stock and ornamental plants present a special and important problem in quality as it affects loss, since a single diseased plant may cause condemnation of large numbers of plants under quarantine laws, and since minor blemishes may render ornamental plants unsaleable.

The phytopathologist may often be required to interpret the partial and joint effects of two or more concomitant loss factors. There is danger in overstressing the factor which is most obvious, most recent in appearance, or with which the investigator is particularly concerned. Such cases can usually be resolved by measuring the effect of each factor separately and then combined, aided, for example, by the use of crop varieties or pesticides that are specific control measures for one or another of the concomitant loss factors. Alternatively, statistical treatments of disease intensity and loss data will often serve properly to attribute to each factor its share of the combined damage.

#### VI. HOW DOES ONE GO ABOUT MEASURING SICKNESS IN PLANTS?

Basically, the problem of plant disease loss appraisal consists of measuring disease intensity and translating this into loss. In this section we are concerned with the methods of measuring and recording disease intensity, while the following section deals with disease intensity-loss relationships. By disease intensity is meant the amount of disease present in a plant, in a field, or in a geographic region, without reference to the damage caused.

##### *A. The Methods Depend upon the Purpose*

Measurements of disease intensity are usually made for either scientific or economic reasons. When measurements are used as an aid in research, as for example, in discriminating between a number of alternative control practices or in comparing the disease reactions of a number of varieties of a crop, it may be necessary that the measurements be highly precise and, as a consequence, time-consuming and costly. Alternatively, if the objective is to determine the economic impact of a disease, it may be quite impractical and unnecessary to use the refinements of disease measurement that are required for research purposes.

##### *B. Measuring Sickness in the Individual Plant versus That in the Population*

Intensity of plant disease, as understood here, is a function of the average degree of sickness in the individual plant and of the prevalence

of affected plants in the population. Where destruction of the individual plants or of their commercial parts is total, as in the head smuts of small grains, it suffices to know the percentage of diseased plants in the population. More commonly, we must deal with varying degrees of destruction in the individual plant, combined with varying percentages of affected plants in the population. And when a disease can affect various organs of the plant in different, harmful ways, as in fire blight of pome fruits, measurement of disease intensity becomes quite complex, although nonetheless possible and necessary.

### *C. Methods and Aids for Determining the Amount or Intensity of Plant Sickness*

#### *1. Number or Per Cent of Diseased Plants, Organs, or Tissues*

When diseased plants or plant parts are total losses and not partial losses, or when all diseased plants or plant parts are partial losses to the same degree, counts of diseased plants or plant parts and conversion of the counts into per cent give accurate measures of disease intensity. Whenever its use is valid, the recording of disease intensity as a per cent of plants or organs affected has the distinct advantages that it is uniform from one worker to another, provided a diseased plant or organ is properly defined and that the definition is easily understood by all.

This method of scoring disease intensity is most useful and reliable in dealing with: (a) diseases in which the entire plant is killed, with few plants exhibiting partial loss, as in *Fusarium* wilt diseases of cotton and other crops, barley stripe, and damping-off of seedlings; (b) cases in which diseased plants, while not killed, are all injured to approximately the same degree, as in virus diseases of vegetatively propagated plants, excluding current-season infections; (c) instances in which the per cent of affected plants is well correlated with the degree of injury, as with corn smut; (d) diseases in which plants or organs, even if lightly affected, are total losses from the commercial standpoint, such as crown gall of nursery stock, or ear smut of sweet corn; (f) cases in which diseased plants or tissues are so rare that differences in degree of infection have little statistical significance.

A good device, where plants or organs differ in degree of attack, is to record the number of plants or organs in each of several disease per cent classes, as: 0-10%, 10.1-20%, . . . 90.1-100%, and reduce this to a single numerical expression of disease intensity. Horsfall (1945) has pointed out the advantage, in this case, of using classes based on the ability of the human eye to discriminate differences, such as the series: 0-3, 3-6, 6-12, 12-25, 25-50, 50-75, 75-87, 87-94, 94-97, and 97-100% disease.

With leaf-cast diseases, the estimated per cent of defoliation is a promising measure of disease intensity that has been too little used. Per cent of defoliation is frequently well correlated with intensity of disease on leaves that have not yet dropped.

## 2. *Descriptive Scales for Evaluating the Amount of Sickness*

The simplest type of descriptive scale, which unfortunately, is still frequently used, is to grade disease in three or more classes under such terms as "light," "moderate," and "severe," and sometimes, to make matters worse, the descriptive word is omitted and the undescribed classes are simply numbered or assigned symbols. Such scales may be meaningless to workers other than the ones who devised them, since "moderate" disease in a region or season in which the disease is very prevalent may correspond to "severe" disease in a year or location with less abundant disease.

Descriptive scales can be useful if the grades are realistic, well described, usable in practice, and comparable from one worker, location, or season to another.

A device that is widely used, with modifications, is McKinney's (1923) "infection index." He used it originally to summarize infection of wheat seedlings by root rot diseases. Each seedling was classified in one of five classes, from healthy to severely diseased. Each class was given a numerical rating, in this case: 0.00, 0.75, 1.00, 2.00, and 3.00 respectively. Then,

$$\text{Infection index} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of inoculated plants} \times 3}$$

The factor 3 was used in the formula because that was the rating of the maximal disease category, while the factor 100 converts the final rating to a basis ranging from 0 for no disease to 100 where every plant is diseased to the maximal extent.

When the class rating is expressed in per cent instead of arbitrary numbers, the disease index may be simplified to the form:

$$\frac{\sum (\text{Class rating } (\%) \times \text{class frequency})}{\text{Number of plants or organs examined}}$$

which gives a mean value for disease intensity in per cent, as of leaf area involved in disease.

The widespread use of the McKinney index, in original or modified form, testifies to its value. It reduces a disease intensity complex to a single expression that is open to statistical analysis on the basis that

"although the estimates are not necessarily in direct linear relation to the amount of fungus present . . . they are reducible to a linear function of this amount" (Marsh *et al.*, 1937).

### 3. *Logarithmic Versus Arithmetic Scales of Disease Intensity*

Horsfall and Heuberger (1942) used the McKinney index for measuring the target spot of disease. Later Horsfall (1945) showed that it lacked adequacy (1) because the grades were too wide and (2) because it ignores the visual acuity of the human eye which sees in logarithms according to the Weber-Fechner law. Accordingly Horsfall and Barratt (1945) devised a new logarithmic scale to make realistic the logarithmic feature of visual acuity. This scale shifts emphasis at the 50% point. Below 50% disease, the eye discriminates on the basis of diseased tissue. Above 50% it discriminates on the basis of healthy tissue not yet diseased. Hence, the scale must be balanced around the 50% point. For convenience the scale as published is based on a ratio of 2 except for the upper and lower ends which of necessity must include the residues. Ignoring fractions the scale is 0-3, 3-6, 6-12, 12-25, 25-50, 50-75, 75-87, 87-94, 94-97, and 97 to 100. Thus, the scale reads the diseased tissue in logarithmic units below 50% and healthy tissue in the same units above 50%.

The subcommittee on Disease Measurement of the British Mycological Society (Anon., 1947) has developed the scale shown below for potato late blight. Empirically, it does for the lower part of the scale

Notation	Degree of Disease Intensity
0.0	Not seen in field.
0.1	Only a few plants affected here and there; up to 1 to 2 spots in 12 yd. radius.
1.0	Up to 10 spots per plant or general light spotting.
5.0	About 50 spots per plant or up to 1 leaflet in 10.
25.0	Nearly every leaflet with lesions; plants still of normal form; field may smell of blight but look green, though every plant affected.
50.0	Every plant affected and about one-half of leaf area destroyed; field looks green, flecked with brown.
75.0	About three-fourths of leaf area destroyed; field looks neither green nor brown. In some varieties the youngest leaves escape infection, so that green color is more conspicuous than in varieties like King Edward, which commonly shows severe shoot infection.
95.0	Only a few green leaves remaining, but stems green.
100.0	All leaves dead; stems dead or dying.



essentially what Horsfall and Barratt proposed but it does less well for the upper part of the scale. Nevertheless, it is a well devised and useful descriptive scale which should result in uniform, comparable disease records from different observers, locations, and seasons.

The value of such a scale is enhanced if accompanied by photographs or drawings illustrating the several grades. Since the stage of development of a plant at the time of its attack with a given intensity of disease is important in determining the amount of loss, good use can be made of comparison scales, one for disease intensity, the other for growth stage of the plant.

#### 4. *Disease Intensity Standards*

A high degree of uniformity in rating disease intensity is possible when use is made of visual standards, including photographs, drawings, or preserved specimens, representative of each of a series of grades of disease intensity. A few of these are available, but many more are needed for uniform scoring of diverse plant diseases, so that each observer may know what others mean by their disease classes, so that we may know how severe is "severe."

The first of these that has come to the writer's attention was the pictorial cereal rust scale of the Australian, Cobb, in 1890-94. This consisted of diagrams of five degrees of rustiness, of from 1% to 50% leaf coverage by rust pustules. In slightly modified form, it was adopted by the U. S. Dept. of Agriculture in 1922 and has been widely used by cereal pathologists, plant breeders, and agronomists in the United States. Other comparable diagrammatic cereal rust standards with further refinements have been developed in Canada, Russia, and Spain (Salazar, 1954). Large and Honey (1955) have published diagrams for potato scab. Chester (1950) fully discusses such standards.

Pioneer work in devising disease intensity standards was done by Tehon (1927) and Tehon and Stout (1930) in connection with their plant disease surveys of Illinois. They have furnished excellent series of standards, in the form of line drawings, illustrating disease intensity grades for *Septoria* leaf spot of wheat, halo blight of oats, cherry and plum leaf spots, diffuse and spot types of apple scab, apple blotch, the leaf phase of apple black-rot, and bacterial spot of peach leaves.

#### 5. *Correlations of Different Expressions of Disease Intensity*

It would be very helpful in disease appraisal if two or more expressions of disease were well correlated one with another. If there should

be a high degree of correlation between root decay and some above ground symptom, for example, some of the labor and time involved in digging up and examining roots would be saved. If two observers should report intensities of a given disease in terms of two different expressions of disease that are well correlated, a valid comparison of the results could be made.

It seems very reasonable to suppose that there often is a regular correlation between per cent of plants affected, per cent of organs per plant affected, and degree of infection per organ. Whenever the effect on one organ is the direct result of disease in another organ, a high correlation between the two may be expected. The phytopathological literature contains many examples of such correlations, such as those between per cent of dead leaves and number of lesions in tomato defoliation disease (Horsfall and Heuberger, 1942), between per cent of plants infested with nematodes, number of nematode galls per plant, and nematode population in the soil (Godfrey, 1934), between injury to tomato vines and fruit-rot from late blight (McNew, 1943), and between spray injury of leaves and preharvest drop of apple fruits (Lewis, 1944).

There are other instances, however, where such correlations do not exist. In the case of apple blotch, for example, there is independent variation in the amount of disease in leaves, twigs, and fruits, among different apple varieties. Apple bitter-rot shows the same situation. With diseases such as these, the several organs must each be appraised, since the amount of disease in one type of organ may give no valid index of the amount in another organ.

## 6. *Forest Disease Appraisal*

This subject has been highly developed in forest pathology, having become a leading phase of forest mensuration. Since it is extensively treated in forestry text and reference books, it is not fitting to give it more than passing attention here.

Wood decay is the leading problem and one in which appraisal is difficult because the injury is largely hidden from view. Direct examination to determine the amount of decay within standing trees is costly and impractical except on a sampling basis, yet it is necessary to know the approximate amount of decay in order to determine value of the timber and optimal cutting time.

The presence of fungus fruiting bodies on the surface of tree trunks is not very helpful since these develop only after decay is well advanced. There are other, useful correlations, however. With top or trunk-rot of oak, Hepting and his associates found a good correlation between wood

decay and rotten branch stubs, surface injuries, and blind knots on the bole. In the case of butt-rot of oak there is a high correlation with fire wounds. A formula relating age and width of the wounds to the amount of butt cull has been derived and used both for determining the amount of cull at the time of surveying and for predicting its amount in the future. A very practical use of correlations is seen in the analysis of tree rings to determine the occurrence and severity of defoliation and other plant injuries in earlier years.

For direct examination of the internal condition of trees, use has long been made of the increment borer, which extracts a pencil-like core of wood, radially from bark to center of the tree, giving an index both of tree growth (annual rings) and amount and type of decay. Among new methods of internally sampling trees are the use of X-rays and radiographs; it is possible that radar might be used for the same purpose.

#### *D. Integrating Disease Intensity Data*

Having measured disease on individual plants, the readings must be integrated for numerous purposes.

The McKinney index has many uses, among which are disease surveying, evaluation of disease in different crop varieties, and evaluation of the efficacy of fungicides and other means of disease control. In using the indexes, judgment is needed in assigning arbitrary ratings to the several disease classes. Where possible, each class rating should reflect the relative intensity of disease or damage in comparison with ratings of other classes. Class ratings of 0, 1, 2, 3, and 4, for example, would be most appropriate if plants or organs in class 4 have four times the disease intensity of those in class 1, twice as much as those in class 2, etc. If care is used in assigning the class ratings, with absolute disease intensity properly considered, the indices themselves will have absolute, not merely relative, value. A logarithmic series of class ratings might often be preferable to an arithmetic one.

Tehon (1927), in statewide surveys of cereal disease, used the following formula for determining average intensity of disease in a field, expressed as per cent:

$$\frac{\text{Class rating (in \%)} \times \text{culm frequency in each class} \times \% \text{ infected culms}}{\text{Total number of culms examined.}}$$

A similar rating was used for fruit diseases. This method gives highly precise and accurate disease intensity estimates. Its chief disadvantage, according to Horsfall (1930), is that it is very laborious and time-consuming. Yet this may not be a serious disadvantage, for Tehon has shown

that the method can be used on a statewide basis, year after year. The formulas suggest more effort than is actually required in many cases. With general outbreaks of some diseases, such as cereal rusts or apple scab, prevalence is usually 100%, which can be easily ascertained. Diseases such as smuts can be quickly estimated by simple counting. The time spent in traveling from one field or orchard to another is such a large element in the survey cost that a fairly thorough examination at each stopping point is justified. However, if the method could be simplified without undesirable loss in accuracy, this should be done. One method of simplification which deserves consideration is the use of correlations. If a constant relationship can be shown, for example, between per cent of trees affected, per cent of affected organs per tree, and degree of attack per organ, then all of these values would not need to be determined independently.

In extending disease intensity estimates to embrace larger regional units, such as counties, states, provinces, or nations, the method used by most workers is a modification of the McKinney disease severity index, with the form:

$$\frac{\Sigma (\text{field rating class} \times \text{acreage in class})}{\text{Total acreage}}$$

The field ratings are usually classified in a series of grades, from 0 to 100% disease intensity, but arbitrary grade values could also be used. Naumov (1924) in Russia, Ducomet and Foëx (1925, 1928) in France, and Yoshimura (1954) in Japan have described methods of summarizing disease intensities which differ in detail but not in principle from those given above.

### *E. Methods of Sampling and Surveying*

For plant disease appraisal data to achieve full usefulness in relation to economics, we must have representative cross-sections of the disease hazards involving whole counties, states, or nations. Such data can best be obtained by plant disease surveys—planned and uniform samplings throughout the areas involved.

#### *1. Organization and Planning of a Survey*

Plant disease surveys should have definite objectives, their objectives should be clearly related to useful application of the results, their methods should be adapted to the specific objectives, and they should be sufficiently thorough to permit reliable conclusions. At times it may



be desirable to study very thoroughly a limited number of fields while in other cases it may be useful to have less precise data from many random samples scattered over a broad area. Sometimes the two methods may be combined. The degree of thoroughness that is desirable, yet economical, depends on the objective. In some cases, data on presence or absence of a disease are sufficient; in others, it may be necessary to determine, with greater or lesser accuracy, the concentration of disease present. Some diseases, such as the cereal rusts, affect great acreages rather uniformly, and here fewer samplings are needed than with diseases where occurrence depends more on local environmental or agricultural conditions. General utility surveys are broad, less intensive, and less exact than special purpose surveys, such as those designed to aid plant disease research.

## 2. *Kinds of Sampling*

We distinguish crop (or commodity) sampling and opinion sampling, both of which are useful in plant disease appraisal. The former consists of evaluating a part of the crop, before or after harvest, and of considering the findings as evidence of the quantity and quality of the whole. The sample may be a few plants in a field, a few fields in a county, a few counties in a state, a few states in a region, or a combination of these. In opinion sampling, which is illustrated by the U. S. Crop Reporting Service, the sample is a part of the human population and the data obtained consist of the opinions of the people in the sample regarding any question asked them.

Several methods of crop or opinion sampling are recognized. Random sampling is illustrated by appraising a crop field at every  $n^{\text{th}}$  mile indicated on an automobile speedometer. Area sampling might involve examining all fields in random areas. Stratified sampling consists of sampling each element in a complex, in proportion to the known prevalence of that element in the complex, for example examining 10 wheat fields for each barley field if it were known that the wheat acreage is 10 times the barley acreage. Finally, in purposive sampling, all or nearly all of the population having narrowly specified characteristics is sampled, as in disease appraisal of the fields of all growers of certified seed potatoes in a State.

## 3. *Nature of the Sample*

The type, size, and number of samples, and the time to take them vary so widely with the purpose of taking them and the physical and biological circumstances that here we must limit the discussion to some



of the basic principles. The subject is treated in greater detail in the writer's monograph on plant disease losses (Chester, 1950).

Of the factors which determine the time, number, size, and type of samples, two are outstanding and diametrically opposed—reliability and economy. Neither can be increased except at the expense of the other. The preferred schedule of sampling must be a compromise which avoids the expense of increasing accuracy beyond the least degree that will give a practical, reasonably satisfactory answer to the problem at hand. The optimal size and number of samples varies with crop, disease, environment, the degree of skill and bias of the appraiser, accuracy of the appraisal methods, and other factors, which requires a thorough study of the disease situation and its variability before one can determine the optimal size and number of samples.

The more disease present and the more uniformly it is distributed, the fewer samples are required for a given degree of reliability. Usually, air borne diseases are more uniformly distributed than those that are soil-borne or are disseminated by other agents than the wind. The more the host plant is uniform genetically, the more uniform will be the distribution of disease, as a rule. Of two alternative methods of sampling, of equal reliability, the more rapid and economical should be chosen. This underlines the value of a comparative study of appraisal methods before adopting any one.

If one can decide the degree of error that can be tolerated in sampling and surveying, then it is a straightforward mathematical problem to determine the minimum size and number of samples that will yield results within the tolerable error. This is standard practice in timber cruising. A good phytopathological illustration is Fernow's prescription for sampling potato stocks of varying disease content, showing, for example, that with 3% disease in the stock, a 400-tuber sample will reveal the disease content within 1% error, with odds of 10:1. If the odds are 30:1, the sample would have to consist of 2735 tubers (Fernow, 1944; Chester, 1950, p. 263). Sampling practices for determining crop yields, which are well developed, are often applicable to plant disease appraisal.

#### *4. Procedures in Sampling and Surveying*

Plant diseases and their effects are often quite irregularly distributed through a field or from one field to another. Diseases also often show the well known border effect, with a greater or lesser disease intensity at the margin of a plot, field, or region. Disease appraisers, unless they have some means of ruling out the personal factor, tend to select samples

that are not truly representative, from more heavily diseased areas or from the "best" of a field or fields of a region. To avoid this error, ingenious methods of obtaining random samples have been devised, and comparable methods should form part of regular sampling practice.

Suitable random samples are obtained by observance of a few principles. Avoid border effect by working well away from the edges of fields or regions of infestation. Use mechanical devices to eliminate subjective error, such as taking samples at measured intervals along a compass line. Distribute the samples widely over the area being sampled. Make use of mechanical, nonsubjective aids such as sampling the plants within a wire loop thrown at random out into a field, or the grain trier which combines many small samples into a composite whole.

In exceptional cases, nonrandom sampling may be desirable. In a survey for rare or new diseases, for example, with emphasis on discovery rather than measure of prevalence, it would be justifiable to concentrate attention on farms that are uncared for or abandoned—where no effort is made to control disease—or on botanical gardens where there is a rich collection of unusual species or varieties of plants, and plants that are recent imports. The whole problem of sampling reduces itself to the need for using common sense and native ingenuity rather than rule of thumb.

When sampling is extended to wide areas it becomes necessary and useful to adopt "shot-gun" methods. Among these are roadside appraisal, without field sampling, which has been used successfully in surveying for Texas root rot, among other diseases. In this case, effort must be made to eliminate the error caused by border effect. Airplane surveying is especially useful with those diseases that show their destructiveness from a distance (see Colwell, 1956). Good opportunities in plant disease appraisal lie ahead in use of color photography in combination with aerial surveys. Neil Stevens (1945) to whom we owe so many original suggestions on surveying practice, has stressed the value of making greater use of the long-distance telephone as an adjunct to surveying, that is cheaper than the time and gasoline used in travel. It is a method that deserves more extensive use.

Finally, in appraising plant disease and its consequences over broad areas, an important aid is the use of opinion surveys that are so devised and weighted as to give a reliable cross-section of area and to insure competence of data sources. The Master Sample Plan in Iowa State College and, on a smaller but perhaps equally reliable scale, the Doane Agricultural Service sampling program illustrate means of obtaining average opinion about plant disease occurrences and losses; these are

weighted in such a way as to insure that each element in the human sample will be proportionate to its representation in the total population.

## VII. HOW MUCH SICKNESS CORRESPONDS TO HOW MUCH LOSS?

### *A. The Disease Intensity-Loss Ratio*

Having determined the intensity of plant disease, it becomes necessary to establish the numerical relationship that exists between disease intensity and the loss produced, the second major step in plant disease appraisal. We are only led into error if we conclude that because a disease is abundant, a high loss necessarily results, or the reverse of this. Judgment or intuition cannot be trusted; we must learn from investigations the amount of loss associated with given disease intensity.

Such investigations are of two classes; statistical or historical methods may be used, or the experimental approach may be followed. Here the discussion is limited to the latter; a discussion of statistical and historical methods for relating loss to disease intensity will be found in Chester's work (1950).

### *B. Greenhouse Infection Experiments*

This method consists of infecting certain plants with disease, under greenhouse conditions, leaving others uninfected or protecting them from infection, measuring the disease intensity, and comparing crop yields. This method has the advantage of control over environment and disease situation. It has the disadvantage of being performed in an environment that is abnormal, compared with field conditions. It is a useful method but should be supplemented with field tests in many instances.

### *C. Field Plot or Bed Infection Experiments*

In using this method, disease is introduced into plants that are growing under normal cultural conditions, and the yields from the diseased plants are compared with those from comparable plots of uninoculated, healthy plants. The chief advantage of the method lies in the normal growing conditions under which the experiment is conducted. The disadvantages are lack of control of numerous environmental and pathological factors, among which is the natural occurrence of disease in the plots intended as healthy controls. In both greenhouse and field experiments, standard pathological and field experimental methods are used, including an approved plot design that will permit statistical analysis of the results.

#### *D. Plantings from Diseased and Healthy Propagating Materials*

In this case, differences in disease occurrence and intensity result from using seed or vegetative reproductive materials that are naturally or artificially infected. Examples are the use of smut-infested grain seed and of virus-infected potato tubers.

#### *E. Comparison of Yields of Rogued and Unrogued Plantings*

To secure comparable diseased and disease-free plots in the presence of natural infestation, diseased plants may be removed from one, and healthy plants from the other. This technique, which is particularly useful with virus diseases, has greatest value when an excessive planting rate is used; and the roguing, with additional thinning, leaves the healthy and diseased plantings—with similar stands—with desired degree of uniform spacing.

#### *F. The Cultural Method*

This involves a comparison of yields of relatively diseased and healthy crops, the disease occurring naturally, with the degrees of disease being due to differences in cultural conditions, such as different methods of soil management. Studies by this method are subject to serious error, owing to the fact that the cultural differences have direct effects on yield levels in addition to their indirect effects in increasing or decreasing disease. Yet in some cases this source of error can be minimized, and in any case data obtained by this method are useful in confirming the results of more accurate experimental procedures.

#### *G. The Individual Method*

This procedure consists in selecting from a planting a given number of diseased plants and a like number of healthy plants, assessing the amount of disease, and comparing yields. The method has the advantage that it may be applied to any nonexperimental planting in which any ratio of healthy and diseased plants may be found. It is standard procedure with diseases that require many years to develop to the stage in which the observer is interested, as in forest wood-decay appraisal. The individual method is most useful and reliable in appraising diseases in which disease differences are due primarily to chance and not to differences in environment or genetic constitution of the plants, which might in themselves affect yields. The topographical method is a variant of the individual method in which the samples, instead of being single plants or small plant groups, are more extensive populations, differing in disease intensity because of environmental factors associated with differ-



ences in terrain or because of differences in exposure to disease inoculum, although comparable in other respects. While useful in some cases, the topographical method is subject to criticism that variations in terrain result in differences in yields quite apart from the effects of disease.

#### *H. Comparison of Fields with Different Amounts of Natural Infection*

This is an extension of the individual method, comparing fields rather than individual plants in a single field. A limitation is that different amounts of disease in different fields can result from environmental factors that in themselves influence yields. The method is most valuable with those diseases that occur by chance and are not unilaterally associated with ecological factors of yield significance. The relative lack of accuracy of this method is compensated for, in considerable degree, by its extensiveness, giving the observer a picture of loss over a broad area instead of in a few experimental plots. It is particularly useful in confirming and extending the results of more intensive studies.

#### *I. Comparison of Yields of Disease Resistant and Susceptible Genotypes*

This involves a study of disease-loss relationships using populations having individuals or lines that differ in their disease attack owing to genetic differences in disease susceptibility. It involves comparing yields of resistant and susceptible crop varieties, selections from a single crop variety, or segregates from hybridization. It is convenient to use at the same time and with the same materials as in plant breeding experiments. The most serious source of error lies in the fact that the different genotypes may differ in inherent yield capacity as well as in disease reaction, so that yield alterations in the presence of disease may not be strictly the result of the disease itself. This danger may be minimized by (1) use of large numbers of genotypes to offset errors caused by certain of them, (2) use of genotypes that are very similar except for disease reaction, or (3) correcting for genetic influences on yield using data from yield measurements of the genotypes in the absence of disease.

#### *J. Comparison of Yields of a Crop with and without Protection with Pesticides*

This major method of determining the amounts of loss caused by given intensities of plant disease is basically a comparison between yields of two plots of the same disease susceptible crop variety, exposed to disease, in which the plants of one plot have been protected from infection by a pesticide. Standard field plot techniques, with approved plot design, replications, consideration of border effect, and analysis



of the significance of the data obtained, are integral elements of this method of disease appraisal.

Much use has been made of this method. Protective seed treatments have been widely used in studying the losses from damping-off. Soil disinfestation has been used in measuring losses from various soil-borne diseases and pests. Sulfur dusting has revealed the degree of damage from cereal rusts. Spraying and dusting of fruits and vegetables have yielded valuable information on losses from various diseases of these crops.

An important source of error in such experiments is the direct effect of the pesticide on yield, apart from controlling disease. This can and should be measured in disease-free situations, with the results used to modify conclusions from pesticide trials. The direct effect of the pesticide may be to increase yields, as with application of copper pesticides to copper-hungry plants, or the reverse, in cases of spray injury. By using graded series of pesticide applications it is possible to plot curves showing the relationship of disease to yield at different levels of disease intensity or at different stages in the maturity of the crop. If those conducting pesticide experiments, for whatever purpose, would regularly make a practice of reporting disease intensities along with yields—a practice which is often omitted—the data would provide a wealth of needed information on disease-loss relationships.

### *K. Artificial Mutilation*

Frequently, the student of loss appraisal will find data, principles, and conclusions which, although derived for an entirely different purpose, bear directly on disease-loss appraisal. This is the case with experiments involving artificial removal of leaves, whether performed in study of leaf-feeding insects, effects of grazing of cattle, fruit size control, plant physiology, or hail injury. Data from such studies aid in understanding losses from many types of leaf diseases.

Some of the principles that have emerged from such studies are these. It is generally true that any defoliation, at any stage in the development of a crop, produces some reduction in yield. In most cases, defoliation and yield reduction are not proportionate, the loss curve rising more steeply with each added increment of defoliation; i.e., the first leaves lost are more expendable than additional ones lost, since surviving leaves appear to function more efficiently than the same leaves on a completely foliated plant. Defoliation is regularly associated with lowered quality in crops, cause and effect showing the same disproportionate relationship with increasing degrees of defoliation as in the case of gross yield. The effects of defoliation differ with the time at which it occurs, commonly being most harmful when the plant loses leaves after its structure

has been differentiated, when it is too late for replacement leaves to form, yet before the foliage has served its photosynthetic function. Loss of leaves is least detrimental under drought conditions, since their loss reduces transpiration. With perennials, the loss of leaves can reduce yields both in the current season and in succeeding ones. Different crops and different varieties of the same crop suffer to different extents from the same amount of defoliation. Loss of leaves frequently has effects that are useful to man, such as accelerating maturity of a crop or facilitating its harvest, and these advantages may largely offset the accompanying loss in yield.

Each of these principles applies to defoliation caused by plant disease. The method of artificial defoliation appears to be a sound, reliable, and conservative approach to an understanding of the losses caused by foliage diseases. It challenges some of our traditional concepts of the damage from disease, confirms others, and stimulates the investigation of some of the little-known aspects of the economics of plant disease.

#### VIII. HOW CAN THESE MEASUREMENTS BE SUMMARIZED AND ANALYZED?

##### *A. Correlation between Disease Intensity and Yields*

Experiments in determining loss from plant diseases are quantitative experiments, requiring the same techniques in experimental design and statistical analysis of the results as are required in other quantitative biological studies where there is more or less uncontrolled variation in repetitions of treatments, materials, and environments. With these precautions taken, it becomes possible to derive statistically and economically significant relationships between disease intensities and yields.

With sufficient data available, these relationships may be simply expressed as coefficients of correlation. This not only brings out the extent to which disease is responsible for yield reductions, but also, by using partial correlations, it is possible to allocate the fractions of total yield reduction due to several injurious factors acting as a complex.

The study of Sallans (1948) on the interrelations of common root rot and other factors with wheat yields is an excellent illustration of this method. From his simple and partial correlations between rainfall, temperature, root rot, insect damage, and yield, it was possible to develop a yield formula which accounted for 77.8% of the variance in yield in terms of these factors.

##### *B. Correlation between Stands and Yields*

If the principal effect of a disease is to thin out stands, the loss caused will be a function of the extent to which stands and yields are correlated. If reduction of stand does not seriously reduce yield, because

of compensation for missing plants by greater productivity of adjacent ones, the disease may be of little significance. Many of the available data on disease intensity, especially from seed treatment experiments, are reported in terms of stand, not of yield, but if stand : yield relationship is known, as well as that between disease and stands, it might be possible to determine losses from disease intensity data on the simple basis that if  $A : B$  and  $B : C$  are known,  $A : C$  can be calculated.

Loss in stand is not usually proportionate to loss in yield. This is because seeds are often planted at excessive rate, with some beneficial thinning, and because when a plant succumbs, the adjacent ones can often benefit by the space made available, thus partially or entirely compensating for the missing plant. The case is complicated when the disease which thins the stand also has residual harmful effects on the surviving plants, as in soreshin of cotton following *Rhizoctonia* damping-off. Such complication can be analyzed by suitable experiments comparing thinning from disease with thinning by mechanical removal of plants.

Agronomic data on stands and yields of corn illustrate the effect of compensation. In an illustrative case, if a normal, complete stand is taken as 100%, a 50% but fairly uniform stand will produce not  $\frac{1}{2}$  as much corn but about  $\frac{2}{3}$  as much, a 65% stand  $\frac{4}{5}$  as much, and 90% stand 97% as much corn as the 100% stand. If the stand is irregularly reduced, with occasional wide gaps between plants, compensation is less effective. Similarly with potatoes, the two plants adjacent to a missing hill compensate for about  $\frac{1}{2}$  of the potential yield of the lost hill. A skip of 2 hills would be  $1\frac{1}{2}$  hills lost, a 3-hill skip,  $2\frac{1}{2}$  hills lost, etc. These relationships are very much influenced by soil, climate, and crop variety. Some crops can compensate for missing plants to a much higher degree than others. With crops without weed control measures, skips in the stand become occupied by weeds which prevents the compensation effect.

### C. Formulas of Disease Intensity-Loss Relationships

The expression "coefficient of injury (or damage)" has been variously used in an attempt to devise numerical expressions of loss in relation to disease intensity. To Gassner and Straib (1936) the "injury coefficient" is the per cent reduction in yield for each week of duration of attack by disease (cereal rust) of a given intensity. Klemm (1940) has used the same term for the expression  $Q = (a-b) 100/a$  where  $a$  = yield of healthy plants and  $b$  = yield of diseased plants. The loss,  $C = PQ/100$  where  $P$  = the number of injured plants. Here  $Q$  is simple loss per cent and  $P$  the per cent of the crop affected.

In Russia, Yachevski (1929) used a term "coefficient of damage" to express the relation of yield under definite conditions of disease ( $b$ ) to yield of healthy plants ( $a$ ), or  $b/a \times 100$ , which gives the per cent of a normal crop remaining after disease has taken its toll. The "coefficient of damage" of Lubimenko (1933) is "that factor by which it is necessary to multiply the degree of damage of the vegetative organs to obtain the actual effect of the damage," i.e., the amount of loss in quantity and quality of the yield. This was used in artificial defoliation experiments with the form

$$\frac{\text{Per cent yield reduction}}{\text{Per cent leaf reduction}}$$

and might equally well be applied to any disease that defoliates plants. To Naumov (1939) "coefficient of injury" is expressed by the formula  $Ry/x$ , where  $y$  = actual yield,  $x$  = amount of yield of diseased plants expressed as per cent of theoretically normal yield, while  $R$  is a constant.

These several types of coefficients can be compared by using an example. Suppose that a crop, which under disease-free conditions would yield 20 bushels to the acre, is subjected to a disease that destroys 30% of the leaves during a 5-week attack and reduces the yield to 15 bushels. Klemm's coefficient would be 25 (i.e., 25% loss), Yachevski's would be 75 (i.e., 75% of a normal crop), and Naumov's would be 0.2 multiplied by some constant, which is not readily comparable to usual loss measures. Lubimenko's coefficient would be 83.3 which relates leaf injury to yield reduction, the latter being expressed as simple per cent loss (25%). Gassner and Straib's injury coefficient would be 5% loss per week of disease attack. Klemm's and Yachevski's coefficients, while simple, fail to consider disease intensity, as do the intensity-loss tables and regressions discussed below.

#### D. Disease Intensity-Loss Tables

Tables in which the approximate amount of loss is given for each of a series of disease intensities, considering also the time of attack, are useful devices for loss appraisal, but except in forest pathology very few of these are available. Best known, perhaps, are the tables relating cereal rust intensity, at various stages of maturity of the crop, to ensuing loss. In forest pathology, there are many useful tables and curves relating loss (cull) to observable indices of wood decay, with stated, permissible degrees of error. The broad applicability of any such tables can be established only after they have been tested over a wide range of species, habitats, and pathological situations.



### *E. Regressions of Disease Intensity on Yield*

Regressions, straight lines, or curves relating disease intensity and yield, have been used very successfully in depicting the losses caused by numerous types of disease. A regression tells us, for each unit of disease intensity, the per cent or amount of resulting loss. With a good regression available, one can read off the amounts directly, having determined the disease intensity, interpolating between experimentally determined points and extrapolating to the 0 and 100% disease points. They give a basis for forecasting losses when time of disease attack is a factor in the regression. Using regressions one can also analyze a series of interwoven factors relating to disease and yield.

The methods of deriving regressions and testing them for significance and for linearity are found in standard works on statistical methods. While regressions are convenient ways of expressing disease-loss relationships, they are only as valid as the data from which they are derived. We have seen that disease intensity-loss relations may vary with variety of crop, strain of pathogen, and environment in which disease develops. The regression of disease on yield derived from data that apply only to certain limited conditions, will itself have application only to those conditions. Fortunately, many of the findings of loss appraisal experiments have rather wide application within the range of error that is permissible for loss estimation.

The literature on plant diseases and their effects on yields contains many raw data that are suitable for analysis by use of regressions, although this has not been done in the published reports. An example is McLaughlin's analysis of Gram's data on potato leaf roll and yields in which a highly significant linear regression was shown, indicating that for every 1% increase in the disease there was a 0.67% yield decrease (Chester, 1950, p. 325). To the student of loss appraisal this suggests the value that lies in a search for such data and their appropriate analysis.

### *F. Extension of Loss Calculations to Large Regions*

In summarizing disease loss data for states or countries, (1) the disease intensities for sub-areas may be averaged, weighting for the crop acreages in the sub-areas, with the final mean disease intensity converted into terms of loss, or (2) disease intensities for the sub-areas are converted into terms of loss, in per cent or in units of production, and the losses are summated or averaged, weighting for the sizes of the crop areas involved, to give a single figure for total loss.

The U. S. *Plant Disease Reporter*, in calculating loss, considers that:



$$\text{Possible production} = \frac{\text{Actual production}}{100\% - \% \text{ loss from disease(s)}}$$

The loss caused by disease is the product of possible production  $\times$  per cent disease loss. To multiply actual production by per cent loss is a fallacy which is avoided here. If disease is causing a 50% loss in a crop that actually produced 1000 bushels, the loss is not 500 but 1000 bushels, because the 1000 bushels actually harvested were only half a potential crop.

As a good, typical example, Horsfall (1930) determined, in the case of *Macrosporium* leaf spot of red clover, that each 1% of leaf spot infection results in a 0.25% hay loss. The mean per cent of infection for the State was estimated by summarizing the individual products of acreages  $\times$  infection per cents and dividing by the total acreage. Then the loss per cent corresponding to the mean infection per cent was applied to the state yield to give the state loss in tons and dollars.

#### G. Application of Loss Ratios to Disease Intensity Data

Most of the recorded plant disease survey data are in the form of disease intensities. As intensity-loss ratios are developed, it becomes possible to go back through the records of disease intensity and convert them into loss estimates. We are just at the beginning of this important application of plant disease appraisal. Few disease intensity-loss ratios have been derived, and fewer still are the cases in which these ratios have been applied to the disease intensity data of past years. Yet such information would be of great value to phytopathology and to the planners of agricultural progress. The derivation of loss ratios and their use in converting recorded disease intensities to loss estimates is one of the most promising methods that can be suggested for obtaining extensive and reliable loss data with minimal labor and cost.

### IX. THE REWARD

This, then, is the wherewithal for plant disease appraisal, so far as we now know. We have considered the many valuable ends that can be served by knowledge of the economic impact of our subject—how sick is the plant, and what this means to our economy. We have seen that the profession of plant pathology itself can progress adequately only if we can recognize, measure, and demonstrate to others the significance of plant disease. In a hungry world, with a shrinking land area to feed and clothe an explosively expanding population, we are derelict in our duty if we content ourselves with vague approximations of the

economic and social significance of plant disease. By developing accuracy in appraisal of plant sickness and the losses it causes, we will be rewarded by being able to be more effective in research and educational efforts, we will provide needed information for agricultural planning and marketing activities, and we will enlist greater understanding and utilization of our efforts and support for them.

In summary, the need for accurate data on plant disease losses is very great; this need has not been met, yet the means for doing so are available. A beginning toward the correction of this neglected opportunity to increase the effectiveness of phytopathological work could be made by efforts along the following lines:

1. Inclusion of the measurement of disease intensity and determination of intensity-loss relationships as a routine part of every formal comprehensive plant disease study.

2. Adoption of the measurement and interpretation of plant disease losses as a major field of research by a group of individual pathologists who have particular interest in the economic consequences of plant sickness.

3. Assembly of published data on disease intensity measurement and intensity-loss relationships as a start toward a relatively complete survey manual on the subject. Included should be data gathered for other purposes, that can be reworked for this purpose. This might well be a joint product of the group indicated in 2, above.

4. Occasional work conferences of the same group for comparing techniques and results and to correct the subjective element in crop loss estimation, i.e., to "calibrate the observer."

5. A course for disease survey workers, including plant disease survey personnel, commercial crop scouts, agricultural economists, and crop census takers, in which the principles evolved under 3 and 4, above, will be organized in such a way as to facilitate the rapid but accurate appraisal of crop loss from disease by these workers.

#### REFERENCES

- Anon. 1958. IX. International Conference on Quarantine and Plant Protection against pests and diseases. Moscow.
- Anon. 1947. The measurement of potato blight. *Trans. Brit. Mycol. Soc.* **31**: 140-141.
- Barratt, R. W., and M. C. Richards. 1944. Alternaria blight versus the genus *Lycopersicon*. *New Hampshire Agr. Exp. Sta. Tech. Bul.* **82**: 1-25.
- Caldwell, R. M., and L. E. Compton. 1939. Effects of leaf rust and artificial defoliation on yield, composition, and quality of winter wheats. *Indiana Agr. Expt. Sta. Rept.* **1939**.
- Chester, K. Starr. 1939. The 1938 wheat leaf rust epiphytotic in Oklahoma. *Plant Disease Rept. Suppl.* **112**: 18 pp.

- Chester, K. Starr. 1950. Plant disease losses: their appraisal and interpretation. *Plant Disease Repr. Suppl.* **193**: 190-362.
- Chester, K. Starr. 1955. Scientific and economic aspects of plant-disease loss appraisal. *Ann. Appl. Biol.* **42**: 335-343.
- Cobb, N. A. 1890-1894. Contribution to an economic knowledge of the Australian rusts (Uredineae). *Agr. Gaz. N. S. Wales* **1**: 185-214; **3**: 44-68, 181-212; **5**: 239-252.
- Colwell, R. N. 1956. Determining the prevalence of certain cereal crop diseases by means of aerial photography. *Hilgardia* **26**: 223-286.
- Ducomet, V., and E. Foëx. 1925. Introduction à une étude agronomique des rouilles des céréales. *Ann. epiphyt.* **11**: 311-411.
- Ducomet, V., and E. Foëx. 1928. De l'appréciation de l'intensité des rouilles du blé. *Bull. Assoc. Intern. Selectionneurs Plantes Grande Cult., Gembloux* **1**: 22 pp.
- Fernow, K. H. 1944. Relation of sample size to accuracy. *Am. Potato J.* **21**: 229-234.
- Gassner, G., and W. Straib. 1936. Untersuchungen zur Bestimmung der Ernteverluste des Weizens durch Gelb- und Schwarzrostbefall. *Phytopathol. Z.* **9**: 479-505.
- Godfrey, G. H. 1934. Indicator plants for measuring soil populations of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. *Soil Sci.* **38**: 3-27.
- Gregory, P. H. 1948. The multiple-infection transformation. *Ann. Appl. Biol.* **35**: 412-417.
- Hartley, C., and A. Rathbun-Gravatt. 1937. Some effects of plant diseases on variability of yields. *Phytopathology* **27**: 159-171.
- Horsfall, J. G. 1930. A study of meadow crop diseases in New York. *Cornell Univ. Agr. Expt. Sta. Mem.* **130**: 139 pp.
- Horsfall, J. G. 1945. Assessing field data. In "Fungicides and Their Action." *Chronica Botanica*, Waltham, Massachusetts. 239 pp.
- Horsfall, J. G., and R. W. Barratt, 1945. An improved grading system for measuring plant diseases. (Abstr.) *Phytopathology* **35**: 655.
- Horsfall, J. G., and J. W. Heuberger. 1942. Measuring magnitude of a defoliation disease of tomatoes. *Phytopathology* **32**: 226-232.
- Johnston, C. O. 1931. Effect of leaf rust infection on yield of certain varieties of wheat. *J. Am. Soc. Agron.* **23**: 1-12.
- Klemm, M. 1940. Ernteverluste, Schadensschätzung und Pflanzenschutzstatistik. *Forschungsdienst* **10**(3/4): 265-275. (*Rev. Appl. Mycol.* **25**: 226-227. 1946.)
- Large, E. C. 1945. Disease Measurement. Field trials of copper fungicides for the control of potato blight. I. Foliage protection and yield. *Ann. Appl. Biol.* **32**: 319-329.
- Large, E. C. 1952. The interpretation of progress curves for potato blight and other plant diseases. *Plant Path.* **1**: 109-117.
- Large, E. C. 1958. Losses caused by potato blight in England and Wales. *Plant Path.* **7**: 39-48.
- Large, E. C., and June K. Honey. 1955. Survey of common scab of potatoes in Great Britain, 1952 and 1953. *Plant Path.* **4**: 1-8.
- Lewis, F. H. 1944. Effect of spray injury on pre-harvest drop of McIntosh apples. *Phytopathology* **34**: 1015-1019.
- Lubimenko, V. N. 1933. [On the coefficients of injury.] *Trudy Zashchite Rastenii* **No. 3**(3): 3-14.
- McKinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *J. Agr. Research* **26**: 195-218.
- McLaughlin, J. H. Unpublished data. Reported in Chester, 1950.

- McMurtrey, J. E., Jr. 1928. Effect of the mosaic disease on yield and quality of tobacco, with suggestions for control. *Maryland Univ. Agr. Expt. Sta. Bull.* **302**: 147-158.
- McMurtrey, J. E., Jr. 1929. Effect of mosaic disease on yield and quality of tobacco. *J. Agr. Research* **38**: 257-267.
- McNew, G. L. 1943. The control of *Phytophthora* fruit rot in tomatoes. *Canner* **96**(17): 12-14, 23-26.
- Mains, E. B. 1930. Effect of leaf rust (*Puccinia triticina* Erikss.) on yield of wheat. *J. Agr. Research* **40**: 417-446.
- Marsh, R. W., H. Martin, and R. G. Munson, 1937. Studies upon the copper fungicides. III. The distribution of fungicidal properties among certain copper compounds. *Ann. Appl. Biol.* **24**: 853-866.
- Naumov, N. A. 1924. [On the question of the possibilities for determining the degree of plant infection by fungous parasites.] *Trudy IV Entom.-Phytopath. Congr. Moscow* **1922**: 217-228.
- Naumov, N. A. 1939. [Rusts of Cereals in USSR.] Moscow and Leningrad, Selkhozgiz, State Printing Office, Sect. Kolkhoz and Sovkhoz, 403 pp.
- Padwick, G. Watts. 1956. Losses caused by plant diseases in the Colonies. *Phytopath. Papers*, No. 1. 60 pp. The Commonwealth Mycol. Inst., Kew, Surrey.
- Salazar, J. 1954. Reconocimiento de las especies de roya del Trigo y estimación de la intensidad de su ataque. [Recognition of the species of wheat rust and evaluation of the intensity of its attack.] *Bol. Inst. Invest. Agron. Madr.* **14**, 30: 248-261.
- Sallans, B. J. 1948. Interrelations of common root rot and other factors with wheat yields in Saskatchewan. *Sci. Agr.* **28**: 6-20.
- Schultz, H. S. 1938. "The Theory and Measurement of Demand." Univ. of Chicago Press, Chicago, Illinois, 817 pp.
- Stevens, N. E. 1933. Plant pathology and the consumer. *Sci. Monthly* **37**: 325-329.
- Stevens, N. E. 1945. Research and plant disease surveys. *Plant Disease Reprtr. Suppl.* **152**: 6-12.
- Tehon, L. R. 1927. Epidemic diseases of grain crops in Illinois, 1922-1926. *Illinois Nat. Hist. Survey Bull.* **17**: 1-96.
- Tehon, L. R., and G. L. Stout, 1930. Epidemic diseases of fruit trees in Illinois, 1922-1928. *Bull. Illinois Dept. Registr. and Educ., Div. Nat. Hist. Survey* **17**, Art. 3: 415-502.
- Valgren, V. N. 1922. Crop insurance: risks, losses, and principles of protection. *U. S. Dept. Agr. Bull.* **1043**: 1-27.
- Wood, Jessie I. 1953. Three billion dollars a year, In "Plant Diseases." U. S. Dept. Agr. Yearbook of Agr. pp. 1-9.
- Yachevski (Jaczewski), A. A. 1929. "A Guide to Phytopathological Observations." A. A. Yachevski Mycological Lab., Leningrad, 237 pp.
- Yoshimura, S. 1954. On the scale for estimating degree of severity of sheath blight by *Hypochnus sasakii* Shirai in rice plant. *Ann. Phytopath. Soc. Japan* **19**: 58-60. (Abstract in *Rev. Appl. Mycol.* **35**: 924, 1956).

## CHAPTER 5

# Tissue Is Disintegrated

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I. INTRODUCTION<sup>2</sup>

The most common of all the diverse symptoms that characterize disease in plants are those that reveal the decomposition of tissues in one or more structures of the host plant. In fact, there are relatively few diseases that do not cause a disintegration of plant tissue in some stage of the pathological process. Rots, blights, and similar diseases involving obvious tissue breakdown were among the earliest maladies of crops recognized by man. Included among diseases of this type are many of the deadliest enemies of crop plants, of which late blight of Irish potatoes is a classic example. The spectacular crop destruction and the aftermath of human suffering brought about by the epiphytotics of late blight in the 19th century have had few parallels in the history of mankind. In addition to the dramatic losses caused by the direct attack of pathogens on growing crops, an inestimable toll is exacted each year by the more insidious rots and decays that affect fruits, vegetables, and seeds. The costs of harvesting, shipping, and marketing such products have risen recently to such an extent that they often exceed the basic cost of the plant product itself and serve to emphasize the need for control of decay losses. Furthermore, the decay of heartwood in standing timber by rot fungi exceeds fire or any other single factor in reducing the volume of merchantable timber in the United States. Thus, the economic importance and broad scope of diseases involving tissue breakdown points to the need for basic knowledge of the mechanisms involved.

In the absence of knowledge of the essential causes of disease, symptoms revealing changes in normal structure and function logically served as the basis for early classifications of plant diseases devised by such men as Zallinger, Adanson, and Fabricius in the 18th century. Basically, this approach reflected the influence of early physicians with their concern for those pathological patterns that would enable them to make diagnoses. Impressed by the importance of diseases involving tissue disintegration, Fabricius (1774) included "decaying" as one of the main classes of disease, and under this classification he listed several "genera" such as rot, putrefaction, and canker. The terms first recorded in those early textbooks were, in most instances, originally coined by the farmers. It is of interest to note that little is known concerning the exact etymological origins of the word "blight." It first appeared in the writings of

<sup>2</sup> The following abbreviations will be used in the text: PME, pectinmethyl-esterase; DP, pectin depolymerase; PG, polygalacturonase; C<sub>1</sub>, a postulated enzyme that converts native cellulose into linear polyanhydroglucose chains; C<sub>x</sub>, a postulated enzyme that hydrolyzes  $\beta$ -1,4-linkages converting linear polyanhydroglucose chains to cellobiose; and CMC, carboxymethylcellulose.

the 17th century as a term used by gardeners and farmers to describe a rapid killing of plants. The word "rot" is more ancient in origin, and synonyms or similar terms appear in the literature of most Scandinavian countries as well as in English writings as early as the 10th century A.D. Of Anglo-Saxon origin, the word "rot" is considered to be derived from the word "ret," used in connection with the process of soaking flax in water with the resulting maceration of the tissues. Other words describing symptoms of tissue breakdown have more ancient origins than those of "rot" and "blight." Canker is considered to be based on the Latin word "cancer" that was modified later in Old French to "chancre." "Anthracnose" has its origins in two Greek words, *anthrax*—meaning carbuncle, and *nose*—meaning disease, and may well be one of the oldest descriptive terms applied to a type of tissue breakdown.

The mechanisms by which certain microorganisms are able to convert healthy plant tissue into a soft or mushy pulp remained almost completely unknown until the latter part of the 19th century. During the 100 years in which plant pathology has existed as a science and particularly during the last decade, our knowledge of these types of disease has been greatly extended. However, in modern textbooks and in recent phytopathological literature on tissue necroses, most of the early descriptive names for necrotic diseases have been retained and as a result they have acquired a certain status of usage as scientific terms in the terminology of plant pathology. It is recognized that such terms may give little insight into specific mechanisms of disease processes.

In general, most of the diseases involving tissue disintegration are caused by organisms that are biologically inferior, if one considers the highly specialized obligate parasites to be among the elite of the parasites. However, the heterogeneity of facultative parasites is great, and the destructive potential of a given microorganism is often totally unrelated to phylogeny. For example, a number of different disease syndromes can be described by the word "rot." However, rots are traceable to so many distinctly different fungi in such widely separated taxonomic groups that the diversity in the nature of rots is much less than the diversity of the microorganisms that induce them. For this reason, the organization of the main portion of this discussion is based on symptomatology and pathological processes in different types of plant tissues rather than on the different types of organisms involved.

The subject area delineated in this discussion of tissue breakdown is not limited to a restricted group of plant diseases. In a broad sense, it is intended to include every pathological situation in which the integrity of normal and healthy tissue is lost. This may involve separation and decomposition of the essential structural components of cell walls, death,

and degradation of living protoplasts with all concomitant complex changes, or combinations of the two.

Major emphasis is placed on processes involved in decomposition of plant tissue by phytopathogenic fungi or bacteria. A detailed discussion of the nature of toxins is not included, although they will be mentioned wherever they are involved (See Chapter 9, Volume II). Disintegration of plant tissue usually connotes degradation of cell walls, and the discussion centers around decomposition of cellulose, pectic substances, and lignin. The structure and chemistry of cellulose, pectic substances, and lignin are, therefore, summarized briefly. Inasmuch as our knowledge of the degradation of protoplasm in plant cells is very limited, this area of information is not emphasized.

## II. THE NATURE OF CELL DISINTEGRATION

The amount of decomposition of plant tissue reflects the degree of disintegration in individual cells. This disintegration can be brought about in two ways: (1) the components of middle lamellae or cell walls are decomposed, resulting in separation and collapse of the individual cells, and (2) the protoplast is attacked directly, with loss of its integrity as a functional unit and injury to cell membranes. These effects may operate simultaneously, and at least one or more components of the cell may disappear completely. Since these two major changes often involve markedly different mechanisms, they are treated separately below.

### *A. Disintegration of the Components of Cell Walls of Plants*

In the majority of diseased plants where tissue is disorganized, the cell walls are affected first. The basic components of the cell wall, cellulose, pectic substances, and lignin as well as non-cellulosic polysaccharides, may be decomposed by enzymes of both pathogenic and saprophytic origin. Knowledge about the nature of these enzymes and about the mechanisms of degradation of these large molecules has been gained more from studies on saprophytic microorganisms than on phytopathogens.

#### *1. Mechanism of Degradation of Cellulose*

In cell walls of higher plants, cellulose is not only the major component but it is also the basic unit of the structural framework. Cellulose is relatively resistant to microbial decomposition, although certain plant pathogens and saprophytes degrade it with ease. As a basis for an understanding of this decomposition of cellulose by phytopathogenic organisms, the structure of cellulose and present concepts of the enzymes involved in its decomposition are discussed.

a. *Structure of Cellulose.* Cellulose molecules consist of long chains of D-glucose residues (1400 to 10,000 per chain) linked together through  $\beta$ -1,4- linkages. These linear chains are arranged in a definite pattern in cellulose fibers (Preston, 1952; Frey-Wyssling, 1953). The relative proportions of crystalline and amorphous cellulose in plant cell walls vary considerably.

The linear chains are bound laterally by hydrogen bonds or other physical forces into narrow thread-like microfibrils which, in turn, are aggregated to form fibrils. Each microfibril may contain from 280 to 800 cellulose chains. The individual chains in the fibrils vary in degree of orientation. Where the orientation is greatest, the tightly packed parallel chains form crystalline areas. Regions in which chains show a more or less random arrangement are designated as amorphous areas. Each linear cellulose chain because of its length passes through several crystalline and amorphous regions. The susceptibility of cellulose to enzymatic degradation may be associated with the relative amount of amorphous cellulose present. In plant cell walls, the intertwining of cellulose fibrils forms a porous lattice-like structure. Submicroscopic spaces between the fibrils form an interconnecting system that extends throughout the cell wall. The spaces are filled with other cell wall constituents which may include lignin, pectic substances, or hemicelluloses in varying proportions.

b. *Nature of Enzymes Involved in Cellulose Degradation.* Cellulose can be completely degraded by a succession of enzymatic actions of various microorganisms (Siu, 1951, 1954; Siu and Reese, 1953). The large cellulose molecules are hydrolyzed by microbial enzymes into simpler and smaller units. These short-chain molecules are then converted into glucose and utilized by the degrading microorganism.

Two theories dealing with the enzymatic hydrolysis of cellulose are based on studies of the destruction of textile products by cellulolytic organisms. According to the unienzymatic theory, a single cellulase may convert native cellulose into glucose by a random cleavage of the molecule. This mechanism was suggested by Whitaker (1953, 1957) on the basis of evidence obtained with an electrophoretically homogeneous enzyme preparation from *Myrothecium verrucaria*.

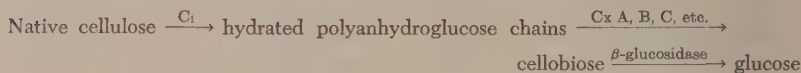
Aitken *et al.* (1956) and others agree that a single enzyme converts cellulose to cellobiose, but regard a cellobiase as necessary for the production of glucose. There is considerable proof that certain wood-rotting fungi such as *Collybia velutipes* and *Polyporus annosus* require a  $\beta$ -glucosidase in addition to cellulase to convert cellulose into glucose (Norkrans, 1957a, b). Cellulose degradation for these organisms presumably occurs in the following steps:





According to the multienzymatic theory, a series of enzymes is required for hydrolysis of native cellulose to glucose. A postulated enzyme,  $C_1$  is presumed to act on native cellulose (Reese, 1956). It is thought that the  $C_1$  enzyme acts mainly on crystalline cellulose which resists the uptake of moisture because of the tight bonding of the chains. Presumably, the action of the  $C_1$  enzyme loosens up the chains so that they take up water prior to their hydrolysis. Thus, the products of  $C_1$  activity are for the most part insoluble. These products are then acted upon by the hydrolytic system of Cx enzymes which convert the linear polyanhydroglucose chains to soluble sugars, chiefly cellobiose and glucose. These sugars are absorbed by the microorganism and utilized internally, presumably by the action of  $\beta$ -glucosidases or by phosphorolytic enzymes.

Certain non-cellulolytic microorganisms easily degrade and utilize cellulose derivatives such as carboxymethylcellulose (CMC), although they are unable to degrade native cellulose (Reese *et al.*, 1950; Reese and Levinson, 1952). This has been attributed to the absence of the  $C_1$  enzyme. Several cellulolytic components, Cx's, have been obtained from culture filtrates of *Myrothecium verrucaria* and *Trichoderma viride* by chromatography (Gilligan and Reese, 1954). These components differed in their rate of movement in the column, in their activity on various substrates, their modes of action, and their behavior in the presence of certain inhibitors. The evidence that there are several Cx enzymes serves to support the multiple enzyme theory. Halliwell (1957), studying the rumen bacteria, has also concluded that several different enzymes are involved in the decomposition of cellulose by these organisms. The following diagram gives a summary of the steps proposed by Reese and his associates for the microbial decomposition of cellulose:



Filtrates of cellulolytic fungi contain an unknown factor that causes an increase in subsequent swelling of cotton fibers in 18% alkali and also an increase in the uptake of Congo red (Marsh *et al.*, 1953). The swelling factor is enzymatic in nature and actually shows many similarities to the Cx enzyme, although it differs from this enzyme with respect to pH necessary for optimum activity (Reese and Gilligan, 1954).

Since the unienzymatic and the multienzymatic concepts of cellulose degradation are each strongly supported by experimental data, the differences in the conclusions reported by various investigators cannot be resolved as yet. However, the multienzymatic hypothesis has been given



very strong support by the recent work of Miller and Blum (1956). These investigators separated multiple components with Cx activity from *Myrothecium* cellulase by zone electrophoresis over long distances. The failure to demonstrate multiple components by moving boundary electrophoresis (Whitaker, 1953) with a purified *Myrothecium* cellulase may have resulted from the use of very short migration distances. In addition to the mounting evidence for the complexity of the enzymatic degradation of cellulose by certain fungi that attack textile products, it is also becoming apparent that microorganisms may differ in the types of cellulolytic enzyme systems that are produced.

The degradation of cellulose in woody tissue containing lignin is mainly caused by a specialized group of fungi in the Hymenomycetes. If one assumes the existence of a distinct C<sub>1</sub> enzyme that is required for conversion of the native cellulose in non-lignified tissue to hydrated anhydroglucose chains, the inability of many organisms that form the C<sub>1</sub> enzyme to degrade wood might indicate that a separate or distinct type of cellulase is formed by the wood decay fungi (Cowling, 1958). However, the inability of textile-destroying fungi with effective cellulolytic enzyme systems to decay wood may also indicate inability to penetrate and spread through the walls of woody cells rather than an inability to utilize the cellulose in lignified tissue.

The mechanisms of cellulose breakdown have been examined with only a few plant pathogens other than wood decay fungi. It is perhaps premature to attempt to relate the findings based on work with textile-degrading fungi to plant pathogens. The fact that some organisms do not degrade native cellulose in culture flasks in laboratory experiments is not evidence that they do not do so in living plants. The cellulose substrates used in the laboratory for studies on cellulolytic enzymes are not necessarily the same as those in the cell walls of either a living plant or a dead and intact one. Furthermore, in nature, certain organisms considered to be non-cellulolytic may produce a cellulase for which the optimal conditions may not be provided in test tube experiments. Very few of the fabric-destroying organisms also are pathogens of herbaceous or woody plants, and as yet, the ability of pathogenic organisms to form cellulolytic enzymes has not been related to their pathogenic potential.

## 2. *Decomposition of Pectic Substances*

The pectic substances are colloidal carbohydrates of high molecular weight that rank next to cellulose in relative abundance in cell walls of herbaceous plants. Pectic substances are the main components of the middle lamellae of cells in parenchymatous tissues of higher plants and

form the main deposits in the intermicellar spaces of the cellulose framework of primary cell walls of herbaceous plants.

Unlike cellulose and lignin, pectic compounds are relatively susceptible to enzymatic attack by plant pathogens as well as by many saprophytic fungi and bacteria.

a. *Concepts as to Structure and Occurrence of Pectic Substances in Plant Cell Walls.* The pectic substances in plant cell walls are of three general types: (1) pectic acid, (2) pectin, and (3) protopectin (Kertesz, 1951; McCready and Owens, 1954). Pectic acid is a linear chain molecule consisting of D-galacturonic acid residues joined through carbon atoms 1 and 4 by  $\alpha$ -glycosidic linkages. The ease with which pectic acid is readily precipitated by calcium and other polyvalent cations to form insoluble salts is attributed to the large number of free acid groups. In the middle lamella, pectic acid exists in the form of calcium and magnesium pectates, and possibly also in an esterified form (Deuel and Stutz, 1958).

Pectin is considered to be a water-soluble methyl ester of pectic acid. Since most of its carboxyl groups are esterified, pectin is neutral and cannot be precipitated by polyvalent cations. A partially de-esterified pectin is referred to as pectinic acid. In plant tissue, pectin is located mainly in the primary cell wall rather than in the middle lamella.

Protopectin is the relatively insoluble parent pectic material that occurs mainly in primary cell walls, particularly in parenchymatous or meristematic tissues. Previously, the insolubility of protopectin has been attributed to linkage of pectin with cellulose or other polysaccharides of cell walls. However, present evidence indicates that protopectin is insoluble because of its large molecular size.

b. *Enzymes that Decompose Pectic Substances.* Many pathogenic and saprophytic microorganisms and certain higher plants produce enzymes that can degrade the various types of pectic substances. The history of pectic enzymes is characterized by a confusion in nomenclature which reflects incomplete knowledge of the structure of pectic substances (Kertesz, 1951; Lineweaver and Jansen, 1951). Although the recent classification of pectic enzymes proposed by Demain and Phaff (1957) has certain advantages, it has not been widely accepted as yet. In order to avoid confusion, the terminology of Kertesz (1951) will be followed, inasmuch as it has been used with only slight modifications by most workers.

Pectin methylesterase (PME), also named pectin esterase and pectase, is the enzyme that catalyzes the hydrolysis of the methyl ester group in pectin and pectinic acids with the release of methyl alcohol. Consequently, pectin or pectinic acids are converted to pectic acids. In

addition to its natural occurrence in certain tissues of higher plants, PME is formed by many different microorganisms.

Pectin polygalacturonase (PG), which has also been referred to as "pectinase," "pectolase," and "polygalacturonase," is considered to be the main enzyme responsible for the degradation of pectic substances. PG catalyzes the hydrolysis of 1,4-glycosidic linkages in the polygalacturonic acid chain of pectic or pectinic acids into polygalacturonic acid chains of smaller molecular size and eventually to monogalacturonic acid. From most sources that have been tested, PG shows highest activity on pectic acids, whereas activity decreases considerably as the methoxyl content of the substrate increases. However, some PG enzymes act equally well on both pectic acid and pectin (Seegmiller and Jansen, 1952; Deuel and Stutz, 1958).

Pectin depolymerase (DP) or pectic acid depolymerase also hydrolyzes the glycosidic linkage of the polygalacturonides such as pectin or pectic acids. However, it differs from polygalacturonase since only large molecules are produced by the splitting of the galacturonic acid chain. Furthermore, the rapid loss in viscosity of pectic substances induced by this enzyme is accompanied by a very small increase in number of reducing groups and galacturonic acid is not produced.

Similar to PG, DP from different sources also varies in activity on esterified and non-esterified pectic substances. The first enzyme of the DP type described was considerably more active on pectic acid than on pectin, and so it was designated as "pectic acid depolymerase" (Mc-Colloch and Kertesz, 1948). However, certain soft rot bacteria produce a DP that acts similarly on non-esterified and esterified pectic substances (Wood, 1955a).

Certain enzymes may have been reported to be DP's because crude culture filtrates or alcoholic precipitates with very weak enzyme activity were used. A weak enzyme preparation may not produce monogalacturonic acid or other low molecular weight intermediates in amounts sufficient to be detected by reducing group measurements or chromatographic methods. It seems desirable to regard any enzyme that hydrolyzes the 1,4-glycosidic linkage in pectic substances as a type of PG. Thus, Demain and Phaff (1957) have classified all enzymes of the PG type into the following categories: (1) endopolymethylgalacturonase I and II, (2) endopolygalacturonase, (3) exopolymethylgalacturonase, and (4) exopolygalacturonase. To distinguish between these enzymes, consideration must be given to: (1) the ability of the enzyme to attack pectin in preference to pectic acid, (2) whether terminal or random hydrolysis occurs, and (3) optimum pH. The five enzymes postulated

for this scheme include three with a random mechanism of hydrolysis (endopolymethylgalacturonase I, endo-PMG II, and endo-PG), and two with a terminal mechanism of hydrolysis (exo-PMG and exo-PG). Under this system, the bacterial depolymerase described by Wood for *Erwinia aroideae* is classified as endo-PMG II. This enzyme with its high optimum pH is thus differentiated from endo-PMG I or DP as it was originally defined (McColloch and Kertesz, 1948; Kertesz, 1951). Examples of endo-PG would be yeast polygalacturonase,  $\beta$ -pectinglycosidase (Schubert, 1954), and polygalacturonase I of *Rhizopus tritici*. Exo-PMG would be exemplified by certain commercial pectic enzyme preparations and exo-PG by the purified form of Pectinol-100D, and polygalacturonase II of *Penicillium expansum*.

Protopectinase is the enzyme system that is said to convert native insoluble protopectin of plant cell walls into soluble pectins and bring about a maceration of cells. We know little more about the exact nature and action of this enzyme, if it is a distinct one, than was known some 60 years ago—when it was first described. However, it appears that protopectinase may be considered to be a type of PG or a PG in combination with PME. The only method for measurement of this enzyme involves determination of the time required for loss of coherence of cells in slices or discs of plant tissues, for which process the specific chemical reactions involved remain to be determined.

If the present concept of the structure of protopectin is accepted as correct, the action of protopectinase cannot be considered as distinct from that of a PG, since protopectin presumably differs from pectin only in chain length. This conclusion is supported by evidence that the protopectinase and the polygalacturonase of *E. aroideae* and of *Fusarium moniliforme*, respectively, cannot be separated on the basis of their major properties (Wood, 1955b; Singh and Wood, 1956). Furthermore, a purified fungal PG or a commercial pectic enzyme preparation containing PG and PME can cause maceration of discs of potato or other fleshy tissues, which is also the action of the hypothetical protopectinase (De-main and Phaff, 1957). However, notwithstanding this evidence and the present knowledge of chemistry of pectic substances, phytopathologists have continued to cling to the term protopectinase.

### 3. Decomposition of Lignin

Next to cellulose, lignin ranks as a major component of cell walls in woody plants, and in certain conifers, 50% or more of the wood may be lignin. Of the different constituents of plant cell walls, lignin is probably the most resistant to decomposition by microorganisms. This is supported by evidence for the long survival of lignin in a relatively unchanged



form, as has been observed in 30-million year-old Sequoia wood and in 800,000 year-old spruce wood (Lawson and Still, 1957). Although lignin degradation may be shown to be a significant aspect of tissue breakdown by microorganisms in certain herbaceous plants, it is at present important mainly as one characteristic phase in the decay of wood by certain wood-destroying fungi known as white rot fungi.

a. *Structure and Occurrence of Lignin in Plant Cell Walls.* To a botanist, lignin may be a specific and well-defined substance, but to a chemist it is still a product of non-specific nature which is determined by analytical methods that are also non-specific (Brauns, 1952). Lignin, unlike previously considered cell wall constituents, is not a carbohydrate or even a simple carbohydrate derivative, but is rather a condensation of one or more types of aromatic nuclei in a complex compound with a high molecular weight. A large part of the lignin molecule is composed of phenylpropane derivatives; it also contains benzene rings which are converted to cyclohexyl rings by hydrogenation (Adler, 1957). Spruce lignin may be formed by the combination of molecules of the isoeugenol type condensed to form dehydrodiisoeugenol type products resulting in chains of indefinite length (Freudenberg, 1957). Some of the phenyl rings may contain a methoxyl group in the *meta* position and a hydroxyl group or phenyl-ether linkage in the *para* position to the side chain. Although there is no doubt that lignin is a high polymer with about 10% of the molecule formed by condensation of phenylpropane units, the manner in which these building stones are linked together is not clear. In at least a part of the lignin molecule the 5-position of the benzene ring is connected with the side chain of another building stone by carbon to carbon linkage. Furthermore, the lignin molecule may be built up of units containing four to five basic lignin building stones.

Schubert and Nord (1957) in summarizing present knowledge of the lignification process propose the following tentative scheme:

carbon dioxide  $\rightarrow$  carbohydrate  $\xrightarrow{\text{aromatization}}$  shikimic acid  $\rightarrow$  p-hydroxyphenylpyruvic acid  $\rightarrow$  primary lignin building units  $\xrightarrow{\text{dimerization}}$  secondary lignin building units  $\xrightarrow{\text{polymerization}}$  lignin

On the basis of many recent contributions, it is evident that rapid advances are now being made in the knowledge of lignin. However, the following statement by Erdtman (1957), at the conclusion of his review of outstanding problems in lignin chemistry, is of interest: "In these days when many lignin chemists appear to believe that the ultimate solution of the lignin problem is near, it may be useful to remember that our belief is greater than our exact knowledge."



In herbaceous plants, lignin occurs mainly in secondary cell walls as the incrusting material in the intermicellar and interfibrillar spaces of cellulose. In woody plants, lignin replaces pectic substances in importance as the compound in association with cellulose. In woody cells, the very thin middle lamella is composed almost entirely of lignin. In the primary walls of wood cells, lignin ramifies through all the intermicellar and interfibrillar spaces of cellulose and forms its own framework organized so that cells retain their shape even after cellulose and pentosans are removed.

b. *Nature of the Enzymatic Degradation of Lignin.* Although lignin is a major component of plants and enormous amounts of this material are eventually decomposed by a restricted number of microorganisms, we still know relatively little about the enzymes involved in the degradation of the lignin molecule. Hampered by the incomplete understanding of lignin chemistry and the lack of specific methods for the isolation and characterization of lignin or its building stones, scientists in this area of research have been faced with the difficult problem of solving a crossword puzzle with too many unknowns and too few clues.

Most of our knowledge of the mechanisms of lignin decomposition is derived from studies on a group of lignin-decomposing Basidiomycetes that are known as the "white rot fungi." In the past, it was assumed that these organisms degraded lignin by a specific enzyme to which such names as "lignase," "ligninase," or "hadromase" (Brauns, 1952; Lawson and Still, 1957) were given.

Evidence for the formation of a specific lignin-decomposing enzyme was based mainly on microchemical tests of decayed wood and the ability of certain of these organisms to utilize partially degraded or, more recently, native lignin as a source of carbon.

Recent investigations have supported the earlier work by Bavendamm and others, indicating that an oxidative rather than a hydrolytic process is involved in lignin decomposition. It is now established that those Basidiomycetes that degrade lignin produce an extracellular polyphenol-oxidase of the laccase type; those fungi that are unable to decompose lignin fail to produce this enzyme or produce it only in small amounts (Fåhræus and Lindeberg, 1953; Higuchi *et al.*, 1956).

The properties of polyphenoloxidase of certain of the lignin-decomposing fungi are unlike other enzyme systems of this type that have been studied previously. It is capable of catalyzing the oxidation of certain lignin-related model compounds associated with native lignin and it is present in culture filtrates of certain white rot fungi growing in a medium with native lignin as the sole carbon source (Gottlieb and Pelczar, 1951).

The advances that are now being made in the knowledge of the polyphenoloxidase systems involved in degradation of lignin promise to

aid in deciphering the problems of lignin structure as well as in contributing to an understanding of the mechanisms of wood decay.

#### 4. *Decomposition of Other Cell Wall Constituents*

a. *Hemicelluloses*. In addition to cellulose and pectin, plant cell walls contain the complex water-insoluble polysaccharides known as the hemicelluloses (Whistler and Smart, 1953). These compounds are important constituents of mature and heavily thickened cell walls of wood, grasses, and seeds. The detailed structure of the hemicelluloses has not been defined as yet. It is known that most represent mixtures of two types of polysaccharides: one is composed of pentose or hexose sugar units and the other is composed of polyuronides containing one or more glucuronic acid units joined in the polysaccharide molecule. Xylan is considered to comprise the major component of this mixture in most plants. In gymnosperms, mannan may replace xylan as a major hemicellulose component. Hemicelluloses also contain arabans and galactans. Xylans are the most abundant pentosans formed in secondary cell walls of plants. Xylans obtained from different types of tissue may vary but they are mainly composed of anhydro-xylose units. Some may also contain L-arabinose. Mannans, which are composed of  $\beta$ -D-mannose residues linked through carbon atoms 1 and 4, are straight chain compounds similar to cellulose and starch. Galactans, like mannans, occur widely in secondary cell walls of straw, seeds, and wood. They are long, unbranched chains of galactose residues joined together through  $\beta$ -1,4-linkages. Araban is associated with pectin in primary cell walls and was formerly regarded as part of the pectin molecule. Araban in apple and peanut is probably built up of a core of L-arabofuranose units linked through carbon atoms 1 and 5. Attached to half of these as side chains are single L-arabofuranose units linked through carbon atoms 1 and 3.

Many microorganisms produce enzymes that degrade hemicellulose, including saprophytic or weakly parasitic species of Mucorales and the wood-rotting Basidiomycetes. The action of hemicellulases presumably converts hemicellulose into pentoses and uronides, but the nature and action of these enzymes have not been studied extensively. Certain of the hemicellulose components may be hydrolyzed directly by cellulolytic enzymes of certain organisms, since it has been shown that xylans can be hydrolyzed by the cellulase of *M. verrucaria* (Bishop and Whitaker, 1955).

#### B. *Disintegration of the Protoplasm*

In disintegrated tissue, not only are the cell walls disorganized, but cell contents are decomposed. The mechanisms of toxic action and the

subsequent disintegration of separate components of protoplasm by plant pathogens have not been studied extensively.

### 1. *Mechanisms Involved in Death of Cells*

In certain diseased plants where a rotting or necrosis of tissue occurs, individual cells are killed as a result of the action of toxic substances elaborated by encroaching microorganisms (see Chapter 9, Volume II for a full discussion of toxins). These toxic substances can cause death of cells that are far removed from invading bacterial cells or fungus mycelium. The process involved is undoubtedly complicated and cells obviously may die because of many diverse interactions, including certain destructive enzyme systems that can be released from the protoplast itself. It has been shown repeatedly that highly injurious substances other than pectolytic or cellulolytic enzymes are produced by plant pathogenic bacteria and fungi. Most of these materials have been demonstrated in culture, and in some cases in infected plants. Few, however, have been obtained in pure form and, with a few exceptions, the chemistry of these toxins and their mode of action remain to be deduced (Brian, 1955).

One possible mechanism by which toxic substances may cause death of living cells is the destruction or alteration of the semipermeable nature of the plasma membrane. The subsequent loss of water and metabolites from the cell may be fatal. Disruption of the plasma membrane also allows unrestricted movement into the cell of molecules of all sizes. These molecules, if toxic, will cause the death of the protoplast. Although it is evidently a physical action, such physiologic disturbances of the cell membrane may be brought about by any number of biochemical actions of the toxic molecule in question. Since the plasma membrane consists of a mixture of lipids and protein chains (Frey-Wyssling, 1953), a toxic entity may destroy the membrane by disrupting one or both of these constituents or by affecting some biochemical process involved in membrane organization.

Toxic compounds that act by affecting the semipermeability of membranes have been reported in phytopathological literature, but their specific mode of action still is not known. The two wilt toxins, lycomarasmin and fusaric acid, produced by *Fusarium oxysporum* f. *lycopersici* cause such a disruption of the plasma membrane in plants (Gäumann, 1956; Gäumann *et al.*, 1952; Bachmann, 1956). Both may also act directly on protoplasm by disturbing the physiological processes within the cell. Whether the above mentioned substances are actually involved in tissue disintegration is not known. Oxalic acid has been implicated as the cause of leakage of water and other nutrients from diseased carrots (Overell, 1952). This may be attributed to an effect on the differential permeabil-

ity of cell membranes. In most foliage blights and many leaf spots, the water-soaking—evident in leaf tissue bordering the lesion—is indirect evidence of injurious effects on plasma membranes with resulting release of cell liquids. It should be recognized, of course, that there is no conclusive proof that cells can be killed by this type of mechanism alone.

Some plant pathogens may be able to kill a cell by secretion of a toxin that acts directly on the protoplasm, disturbing or inhibiting a normal metabolic process of the cytoplasm or the nucleus. A toxic substance may injure protoplasm in any one of several ways. It may coagulate or hydrolyze proteins in the protoplasm, block various enzyme systems in the cell, or serve as an antagonist to some vital metabolite of the living organism.

Two toxic fractions have been isolated from the culture filtrate of *Piricularia oryzae* which causes blight and leaf spot of rice in Japan and other rice growing areas of the world (Tamari and Kaji, 1955). Toxin A is  $\alpha$ -picolinic acid; toxin B, designated as piricularin, is more toxic than  $\alpha$ -picolinic acid and inhibits the polyphenoloxidase system of the host. The actual relationship of these toxins to the death of cells in the necrotic lesions is not known. Both toxins undoubtedly play a role in disease processes, however, since they can be detected in the diseased plants.

The wildfire toxin produced by *Pseudomonas tabaci* is the only phytopathogenic toxin for which the mode of action and chemical nature has been clearly demonstrated (Braun, 1955). When introduced into leaf tissue, this toxin does not cause necrosis directly, but produces a chlorotic spot similar to the halo present around the central necrotic lesion on tobacco leaves infected with the wildfire bacterium. Because of its close structural resemblance to methionine, the toxin acts as an antagonist or antimetabolite of this amino acid, interferes with its utilization in the normal metabolism of the plant, and produces chlorosis. This toxin, in conjunction with other products of the pathogen, presumably contributes to the localized necrosis that is characteristic of the disease.

Death of plant cells due to direct effect on the protoplasm may be brought about by alternaric acid, a toxin produced by *Alternaria solani* (Brian, 1952; Pound and Stahmann, 1951), by the toxin produced by *Helminthosporium victoriae* (Pringle and Braun, 1957), and possibly also by the phytotoxic substance formed by *Phytophthora infestans* (Rönnebeck, 1956).

## 2. Degradation of Components of Protoplasm Following Death

After the cell walls have been disorganized and the protoplasts killed by the action of toxins and enzymes secreted by a facultative pathogen, the invading organism utilizes the contents of the cells for its growth.



Very little is known about this aspect of degradation of protoplasm, although the various carbohydrates, lipids, and proteins present in the cell presumably can be readily digested by the enzymatic activity of most bacteria or fungi.

Fungi and bacteria hydrolyze proteins by enzymes similar to those involved in such mechanisms in higher plants and animals. It has become increasingly evident that the complexity of proteolytic enzymes reflects the innate complexity of proteins themselves. Originally, the proteolytic enzymes were classified into two main groups: the peptidases that hydrolyzed the peptide bonds of di-, tri-, or polypeptides to produce amino acids, and the proteases or proteinases that attacked not only the peptide bonds of peptides, but also hydrolyzed large intact protein molecules into peptides and amino acids. The terms endo- and exopeptidase are now used to distinguish between those enzymes that split bonds adjacent to specific amino acid residues in the protein molecule (endopeptidases) and those that attack only the terminal bonds of a peptide chain (exopeptidases). The latter group of enzymes is diverse and includes some that attack terminal residues of entire proteins and some that split only certain small peptide molecules.

The simple sugars of the cell, such as monosaccharides and disaccharides, as well as the polysaccharides, usually become rapidly involved in metabolic activities of most pathogens. The monosaccharides are utilized directly, while disaccharides are either hydrolyzed to monosaccharides by one of the glucosidases or degraded by a phosphorylative process. One of the main polysaccharides in plant cells is starch, with amylose and amylopectin as its two components (Whistler and Smart, 1953). Amylose is a straight chain molecule consisting of 300 to 1000 glucose residues united by 1,4- $\alpha$ -glycosidic linkages. Amylopectin, a much larger molecule than amylose, consists of glucose chains that are involved in a multiple branching system.

The two main enzymes involved in starch breakdown are  $\alpha$ - and  $\beta$ -amylases. The former attacks the 1,4-glycosidic linkages of both amylose and amylopectin to form dextrans, with approximately 6 to 12 glucose units as the primary product of hydrolysis. In a second phase of the action the dextrans are slowly converted into maltose and some glucose. The  $\beta$ -amylases hydrolyze amylose to maltose. The maltose is then acted upon by maltase, an enzyme produced by many different starch-utilizing organisms, to yield glucose.

Many facultative plant parasites are capable of utilizing starch as a sole carbon source, although relatively little detailed information is available about the nature of the specific enzymes involved for these organisms. Certain saprophytic fungi, including members of the genus



*Aspergillus*, have been studied more intensively than have any of the plant pathogenic fungi. A survey of the biochemical properties of plant pathogenic bacteria as listed in Elliott (1951) indicates that many cannot utilize starch as a sole source of carbon. This inability characterizes many species of *Erwinia* and *Pseudomonas*, whereas most *Xanthomonas* species grow readily with starch as a carbon source.

### III. THE DISINTEGRATION OF DIFFERENT TYPES OF PLANT TISSUES

Although it is difficult to generalize, most plant pathogens are likely to attack specific types of tissues or organs. In the following section the diseases involving disintegration of tissue have been classified according to the kind of tissue affected by the casual organism; pathogenic processes as well as the accompanying biochemical phenomena will be emphasized.

#### A. Decay of Parenchymatous Tissue in Fleshy Vegetative, Reproductive, or Storage Organs

Among the most common diseases of plants are the various rots of fruits, bulbs, tubers, roots, and fleshy green leaves. Two kinds of rots, soft and dry, can be recognized on the basis of the consistency and surface character of the infected tissue.

##### 1. Soft Rots

In the majority of cases, disintegration of parenchymatous tissue of fruits and vegetables produces a soft rot. What happens at the microscopic level has rarely been studied in detail in any soft rot, although more is known about pathological histology or morbid anatomy of bacterial soft rots than about those caused by fungi.

Initial investigations of the mechanism of tissue destruction by soft rotting bacteria provided evidence that certain of these bacteria produced enzymes that could dissolve middle lamellae of the cells of such storage organs as turnips, carrots, and potatoes (Potter, 1902; Van Hall, 1903). However, the classic studies of Jones (1905, 1909) were mainly responsible for the establishment of basic concepts as to the mechanism of tissue breakdown by *Erwinia carotovora* and other soft rot bacteria.

During the past 15 years tremendous advances have been made in our knowledge of the biochemistry of pectic enzymes. Yet, it was only after 1950 that phytopathologists in their publications gave recognition to the possibility that a crude preparation from a culture filtrate may contain several enzymes capable of catalyzing the different reactions involved in degradation of pectic substances. Thus, in much of the literature in this subject area, the maceration of tissue discs has been regarded

as evidence of the action of one enzyme, protopectinase.

Kraght and Starr (1953) first showed that *Erwinia carotovora* produced two distinct pectic enzymes, PME and a PG. A more detailed study of pectic enzymes produced by *E. aroideae* was made by Wood (1955a) who concluded that this bacterium mainly produced a type of PG that was referred to as bacterial DP. Production of pectic enzymes by three common soft rot bacteria was investigated also by Echandi *et al.* (1957) who found that *E. carotovora*, *E. atroseptica*, and *E. aroideae* produce DP. None of these three organisms produced PME. However, it is possible that PME could not be detected because of assay methods not sensitive enough to reveal the presence of the enzyme in small amounts. A previously undescribed soft rot bacterium, *E. maydis*, also secretes an extracellular DP that is similar to the DP of *E. aroideae*. However, both this species and *E. chrysanthemi* form PME (Husain and Kelman, 1959).

In an intensive survey of a large number of phytopathogenic and saprophytic bacteria for pectic enzyme production, assays were made of PME, PG, and two types of DP (Smith, 1958a). Following the terminology of Schubert (1954), the PG was designated as  $\beta$ -pectinglycosidase and the DP as  $\alpha$ - or  $\gamma$ -pectinglycosidase. Those phytopathogenic bacteria tested that were capable of affecting pectic materials produced  $\gamma$ -PG. PME activity was restricted to certain species of *Erwinia*, *Xanthomonas campestris*, and certain cultures of *X. vasculorum*.

Chromatographic analyses of the products of enzyme action indicated that the species of soft rot bacteria tested were capable of hydrolyzing pectin to galacturonic acid and oligo-uronides of low molecular weights (Smith, 1958b). With the exception of only one strain of *E. carotovora*, most of the cultures of other bacteria that failed to produce PME also failed to liberate galacturonic acid. These data indicate that most members of the soft rot group probably form both  $\alpha$ - and  $\gamma$ -PG, and confirm results of Kraght and Starr (1953) indicating that *E. carotovora* produces PME and PG. However, the results differ from the reports by Wood (1955a) and Echandi *et al.* (1957) discussed previously. The differences in the results of these investigators may well reflect variation in specific techniques and media employed as well as inherent differences in the strains used.

In the light of the above results, the chief mechanism of disintegration by soft rot bacteria is the degradation of pectic substances in the middle lamella and cell wall by the extracellular pectic enzymes which these bacteria secrete.

Whether cellulose and hemicelluloses are also degraded by soft rot bacteria is still an unsolved question. Husain and Kelman (1959) exam-

ined culture filtrates of certain soft rot bacteria for the presence of the type of cellulolytic enzyme designated as the Cx enzyme. For three soft rot bacteria, *E. carotovora*, *E. atroseptica*, and *E. aroideae*, negative results were obtained. However, Ammann (1951) found that the strain of *E. carotovora* used in his tests did produce a Cx enzyme. A similar cellulase is secreted by *E. maydis* in culture (Husain and Kelman, 1959).

Other soft rot bacteria may produce enzymes that are cellulolytic as well as those that break down non-cellulosic polysaccharides or hemicellulose. However, there is no conclusive evidence that these enzymes play a role in tissue disintegration by these pathogens and the main disintegrative action of bacterial plant pathogens causing soft rots is presumably exerted on pectic substances. This may explain the apparent inability of soft rot bacteria to attack and decay mature, hardened tissues containing relatively little pectic material and more cellulose and lignin.

Certain basic similarities in symptoms are apparent between soft rots caused by bacteria and those caused by fungi. However, the process of pathogenesis may involve more complex mechanisms for the latter than for the former, including the action of cellulolytic enzymes and toxins on host tissues.

In one of the classic papers of phytopathological literature, De Bary (1886) reported that *Sclerotinia libertiana* caused partial or total dissolution of certain constituents of cell walls. An extract from the infected tissue contained an active principle that could macerate and kill healthy tissue. As the active principle was found to be heat-labile, De Bary concluded that cell wall dissolution was caused by an enzyme produced by the fungus. Although the nature of the toxic action was ill defined, it was on these observations that the first concepts of physiology of parasitism of the facultative parasites were established.

Ward (1888), working on a lily disease caused by *Botrytis* sp., confirmed and amplified the work of De Bary. Ward's paper is a classic in itself and its importance as a highly significant early contribution unfortunately has not been fully appreciated. Although his description of tissue breakdown by *Botrytis* sp. is similar to that made by De Bary, Ward also observed actual penetration of cell walls by the hyphae of the fungus and concluded that a cellulose degrading enzyme was responsible for the dissolution of cell walls by the pathogen. In an attempt to characterize this enzyme, Ward obtained a partially purified preparation from a liquid culture filtrate of the organism and also recovered it from diseased lily tissue. An aqueous solution of this enzyme produced symptoms similar to those induced by the fungus itself, including dissolution of cell walls and middle lamellae and swelling of walls in the initial stages of attack.

Ward's study was the first experimental indication of production of an extracellular cellulase by any microorganism, and it preceded by many years the basic work on microbiological degradation of cellulose. Ward was also well ahead of other early investigators in attempting to demonstrate the existence of an enzyme free of a living cell that could induce a specific reaction *in vitro*.

Brown (1915, 1917) presented conclusive proof that a pectic enzyme was involved in the maceration of plant tissue by *Botrytis cinerea*. An enzyme, present in extracts of spores and mycelium of the fungus as well as in culture filtrates, macerated healthy parenchymatous tissue of various plants. All the enzyme preparations obtained by Brown not only caused cells to separate but also caused the protoplasts to die. Since the active principle in every case was heat-labile and non-dialyzable and the killing principle could not be separated from the macerating enzyme, he concluded that in the case of *Botrytis cinerea* both death and maceration of cells were brought about by an enzyme which he called protopectinase. Brown questioned the concept presented by Smith (1902) that oxalic acid had any role in the killing of cells, inasmuch as *B. cinerea* extracts did not contain oxalic acid in concentrations great enough to injure cells.

Detailed studies on the physiology of parasitism of *Rhizopus nigricans* on sweet potatoes revealed that the rotting of tissue by this organism was also brought about mainly by the action of a pectolytic enzyme (Harter and Weimer, 1921, 1923).

Since the time of publication of Brown's work on *B. cinerea* and the studies by Harter and Weimer, many additional investigators have concluded that pectolytic substances produced by soft rot organisms cause tissue breakdown (Brown, 1936). Many of the most significant contributions in this area have come from the Imperial College of the University of London by Brown, Wood, and their co-workers (Brown, 1955; Wood, 1955b).

Demonstration of the formation of pectinolytic enzymes in host tissues invaded by soft rotting fungi is one essential facet in the ultimate proof that these enzymes are involved in tissue maceration. The absence of pectinolytic activity in extracts of apple tissue decayed by such organisms as *Sclerotinia fructigena*, *S. laxa*, and *B. cinerea* posed an interesting problem in analyzing this relationship. In this instance loss in enzyme activity has been explained on the basis of the formation of inactivating substances, oxidative in nature, that are released from the dead cells after maceration has occurred (Cole, 1956). The pectic enzymes of the causal agent of a "watery soft rot" of fleshy vegetables and fruits, *Sclerotinia sclerotiorum*, have recently been characterized by Echandi



and Walker (1957). Both PME and a PG active at low pH levels were formed *in vitro*. Enzyme activity was inhibited by extracts from healthy tissue of potato and onion, two hosts that are not infected by this decay fungus. The specific nature of these enzyme-inhibiting substances present in healthy tissue of resistant plants and in decaying tissue of certain susceptible plants has not been determined.

One of the unique examples of the high potency of pectinolytic enzymes was demonstrated in studies on the causal factors for softening of pickling cucumbers in brine vats. The presence of high populations of pectinolytic fungi, including certain plant pathogens, on flowers that adhere to the cucumber was related to high levels of pectinolytic activity of the brine and the resulting softening of the cucumbers (Etchells *et al.*, 1958). Cellulolytic activity was also demonstrated for the fungi most commonly found on the blossoms.

Conclusive evidence is now available that certain of the soft rot fungi, including *B. cinerea*, produce cellulase (Reese and Levinson, 1952; Köhlmeier, 1956). One common soft rot fungus, *Sclerotium rolfsii*, which is capable of attacking mature and hardened tissue, has been found to be a strongly cellulolytic fungus. In culture it produces an extracellular cellulase (Cx) that can also be detected in invaded tomato tissue (Husain, 1957). Three fungi (*Botryosphaeria ribis*, *Glomerella cingulata*, and *Phylospora obtusa*) that rot apple fruits have been studied recently for their ability to produce cellulase (Husain and Dimond, 1958b). All three pathogens produced significant amounts of cellulase in culture and degraded different types of cellulose as well as substituted cellulose derivatives. It is now evident that the degradation of cellulose in addition to that of pectic materials is an essential phase of tissue disintegration by many soft rot fungi.

The exact manner in which cells are killed by bacterial and fungal plant pathogens causing soft rots is still unknown. The concept that both killing and macerating can be attributed to pectic enzymes has a number of strong supporters (Brown, 1955; Tribe, 1955; Fushtey, 1957). Evidence for this viewpoint exists in the fact that heat treatment of culture filtrates of certain soft rot organisms destroys both macerating and killing activity. Furthermore, all attempts to separate macerating from toxic effects by chemical and other procedures have been unsuccessful. Also, results with extracts of *B. cinerea* and *E. aroideae* were essentially similar. However, it is difficult to visualize how pectic enzymes alone can act as poisons to living protoplasm. Pectic enzymes may possibly make plant cells more susceptible to other toxic metabolic products by rendering cells accessible to toxic molecules that do not enter intact cells



freely. Another possibility for which there is no direct evidence is that the cells are killed before the enzymes act, in which case pectic enzymes would not be involved in death.

If the pectic enzymes are not accepted as the direct cause of death, the nature of the toxic entity still remains to be determined. Several investigators adhere to the concept that *Sclerotium rolfsii* (Higgins, 1927) and *Sclerotinia sclerotiorum* (Overell, 1952) kill cells by the oxalic acid that they produce. Brown (1936), however, concluded that oxalic acid was definitely not involved in the case of *Botrytis cinerea*. There is no doubt that oxalic acid can damage plant cells if concentrations are high enough. Until more experimental evidence is provided, the possibility still exists that oxalic acid takes part in the collapse of cells invaded by some fungal pathogens.

## 2. Dry Rots

A fungal attack on a dormant storage organ of a plant often causes shrinkage and collapse of the affected tissue with little or no exosmosis of water or other liquids. The surface of the invaded plant part remains dry and often compact and coherent. Although few rots of plants can be classified exclusively as dry rots, various gradations exist between a typical soft rot and a typical dry rot. Typical examples of non-watery rots are "dry rot of potato" caused by *Fusarium caeruleum* and the cucumber scab disease caused by *Cladosporium cucumerinum*. We can conjecture that the dry nature of some of these rots may reflect either the nature of enzymes or toxic substances produced by the fungus or it may indicate a slow rate of growth of the parasite in the host, allowing adequate time for the host to form a defense barrier. Since the exosmosis of liquids from host cells usually reflects injury to the plasma membrane, it is also possible to surmise that certain dry rot fungi may be less toxigenic than the typical soft rot organisms.

Very little data are available on the mechanism of tissue disintegration in these dry rot diseases. It does appear logical to assume that enzymes similar to those described previously for soft rot fungi cause limited tissue disintegration without complete hydrolysis of cell wall materials. It is known, for instance, that the causal agent of scab of cucumber, *Cladosporium cucumerinum*, produces a limited dry rot of fruits only under certain conditions. In the field, the fungus rarely causes extensive soft rot, and disintegration of fruit tissue occurs in very localized spots. Histological studies revealed that not only were the cells disorganized and killed in diseased tissue, but that the complete dissolution of cell walls produced lysigenous cavities (Pierson and Walker, 1954). Cellulose in the cell walls was also found to be altered. In an

investigation of the pectolytic and cellulolytic enzymes of this fungus, production of PG and cellulase was demonstrated in culture (Husain and Rich, 1958). Since no PME was produced and the PG was only weakly active on esterified pectic substances, it is possible that the pathogen advances slowly in the host because of localization of the fungus by a defense mechanism of the host.

### B. Necrosis and Disintegration of Cortex and Phloem in Plant Stems and Roots

Various fungi attack and cause decay in growing stems and roots as well as in the fleshy storage organs of higher plants. The most important diseases in this category are cankers, anthracnoses, damping-off, and root and foot rots.

#### 1. Canker and Anthracnose

A canker is a localized wound or necrotic lesion which is often sunken beneath the surface of the stem of a plant and surrounded by healthy tissue. In most instances, canker diseases involve a disintegration of cortex, phloem, and cambium tissue. Although the term "canker" usually designates symptoms on woody or herbaceous stem tissue, it may also denote necrotic areas caused by bacterial infections on fruits. In a typical stem canker, the attack of a fungus or bacterium first produces a small necrotic lesion followed by corrosion and sloughing away of bark and sometimes even of the outer portion of wood. In a slow growing canker, uniform concentric rings of callus tissue develop as exemplified by European canker of apple trees caused by *Nectria galligena*.

A very destructive canker disease is chestnut blight caused by *Endothia parasitica*. Production of a toxin by this fungus not only brings about the death of phloem and cambium tissue but also results in the deposition of gums and formation of tyloses in the xylem vessels, with a resulting cessation of water movement (Bramble, 1936, 1938). However, *E. parasitica* produces no marked effects on the strength properties of woody tissue possibly indicating low cellulase activity. In contrast, *Strumella coryneoides*, the cause of a serious canker of oaks, can be considered to be cellulolytic since it decays xylem tissue slowly (Heald and Studhalter, 1914). Histological studies on phloem and xylem tissue from trees parasitized by *Nectria galligena* revealed that this organism kills living cells before hyphal penetration (Ashcroft, 1934). The manner of separation of cells in the phloem indicated that pectic substances of the middle lamellae were degraded by the fungus. However, cellulose

and lignin were apparently unaffected and intracellular growth of the mycelium was restricted to wood rays in the xylem tissue.

Although very few studies have been made on the canker fungi with respect to their production of pectinolytic and cellulolytic enzymes, it is logical to assume that all of them that decay parenchymatous and woody tissue produce these enzymes. A pectin-decomposing enzyme is formed by the apple bitter rot fungus, *Glomerella cingulata*, which produces small cankers on the bark of apple twigs as well as a fruit decay (Menon, 1934). This fungus and two other canker-forming fungi, *Physalospora obtusa* and *Botryosphaeria ribis*, form cellulase in culture (Husain and Dimond, 1958b). Of these three fungi, *Botryosphaeria ribis* is the most destructive invader of woody tissue since the other two form only superficial bark cankers. It is of interest to note that *B. ribis* produces much higher levels of cellulase in culture and grows more profusely on native cellulose than either of the other two bark-invading fungi.

Certain fungi that bear their spores in acervuli produce necrotic and sunken ulcer-like lesions on the stems, flowers, or fruits of host plants. Such lesions produced by species of *Colletotrichum*, *Gloeosporium*, and other closely related fungi are characteristic of the diseases known as anthracnoses. The anthracnose and the soft rot organisms differ markedly in that necrosis and tissue disintegration follows invasion by the former and precedes that of the latter. Furthermore, a slimy or watery rot of the entire organ of the plant rarely occurs. This suggests possible fundamental differences in the nature of the injurious substances formed by each type of organism.

In the bean anthracnose disease, the fungus initially forms hyphae inside the cells without killing them; then "secondary mycelium" develops and spreads rapidly among and within the host cells, which then collapse quickly and die (Leach, 1923). The enzymes produced by species of *Colletotrichum* have not been investigated extensively, although *Colletotrichum phomoides*, causal agent of anthracnose of tomato (Ragheb and Fabian, 1955), and *C. lagenarium*, causal agent of cucurbit anthracnose (Etchells *et al.*, 1958), have been shown to produce pectinolytic enzymes.

The type of intracellular growth which these fungi exhibit suggests that cell wall penetration may involve the action of cellulolytic enzymes. The flax anthracnose fungus, *C. lini*, forms cellulase, and flax tissue invaded by this fungus shows decomposition of cellulose (Büdiger, 1952); in contrast, *C. lagenarium* does not form cellulolytic enzymes (Etchells *et al.*, 1958). For the majority of other anthracnose fungi direct evidence for cellulase production is not available.

Pathological symptoms also indicate that the anthracnose fungi produce substances injurious to the protoplasts. The *Colletotrichum* sp.,

causing anthracnose of tobacco, produces a toxic material in culture that induces necrotic symptoms in tobacco or tomato plants similar to those observed on diseased plants in nature (Wolf and Flowers, 1957).

## 2. Damping-off

One of the types of disease to which almost all seed plants are susceptible is the rapid death and collapse of very young seedlings in the seed bed or field. During the critical period immediately following emergence of the delicate seedling from the soil, certain fungi such as *Rhizoctonia* spp. (*Pellicularia*) produce a necrosis of the succulent cortical tissue at the base of the stem. Species of *Pythium* are likely to initiate invasion of the tiny rootlets. Under the impact of either of these two types of attack, young plants may yellow and wilt presaging eventual collapse and death. If a given plant survives, the girdling of the stem at the base or a root rot may stunt it for life. This often happens in "sore shin" of cotton seedlings affected by *Rhizoctonia solani*.

Damping-off may be caused by a large number of fungi. The most important of these are *R. solani*, and species of *Pythium*, *Phytophthora*, *Sclerotinia*, and *Fusarium*. Of the *Fusaria*, *Fusarium moniliforme* is considered to be the most important species.

The young and growing tissues of seedlings contain large amounts of pectic substances, and the main enzymatic process operating during initial invasion of tissues is apparently the degradation of pectic substances by the invading fungi, particularly for species of *Pythium* and *Phytophthora*. This conclusion is supported by data on the production of pectic enzymes by a number of different species of *Pythium* (Menon, 1934; Damle, 1952; Ashour, 1954). In the case of *P. debaryanum*, no PME but a DP is formed. This enzyme acts only on pectin and breaks down the molecule to large fragments without the production of any mono-, di-, or tri-galacturonic acid (Gupta, 1956; Wood and Gupta, 1958).

With the exception of *Pythium irregulare* (Dillingham, 1955) and certain species of *Phytophthora*, including *P. parasitica* (Mehrotra, 1949), fungi belonging to the family Pythiaceae do not actively degrade cellulose *in vitro*. Hence it is doubtful that cellulase plays a significant role in the enzymatic destruction of cortical tissue of seedlings by fungi in this group.

Unlike most Phycomycetes, the damping-off fungi in other classes disintegrate host tissues by means of both cellulolytic and pectic enzymes. Equally as important as *Pythium* as a cause of damping-off is *Rhizoctonia solani* which produces both pectinolytic (Matsumoto, 1923) and cellulolytic enzymes (Köhlmeier, 1956). Another damping-off fungus, *Fusarium moniliforme*, secretes both a PG (Singh and Wood, 1956) and cellulase



(Venkata Ram, 1956). Those pathogenic fungi capable of forming both cellulolytic and pectinolytic enzymes appear to be more versatile with respect to age of plants attacked than those pathogens that mainly form pectic enzymes. Species of *Pythium* which are generally unable to degrade cellulose are rarely associated with tissue breakdown of hardened or mature tissues, whereas both *Rhizoctonia solani* and *Fusarium moniliforme* can cause stem or root rots in older plants.

Damping-off fungi may damage young tissues not only through cell wall decomposition but also by toxic effects. A heat-stable toxic principle that was lethal to young seedlings was found in culture filtrates of three species of *Pythium* (Damle, 1952). Similarly, *Pythium irregulare*, a common parasite of sugar beets, produces a potent toxin *in vitro* (Brandenburg, 1950).

### 3. Root and Foot Rots

A wide range of pathogens affecting an equally wide range of hosts produce rotting of cortical and phloem tissue of roots and basal stem portions of older seedlings or full grown plants. Root rots are caused by different species of *Fusarium*, *Rhizoctonia*, and *Sclerotium* as well as a large number of other facultative parasites that are typical soil inhabitants. Some root rots which cannot be attributed to any single pathogen may involve several microorganisms, sometimes including plant parasitic nematodes. The root rot complex of strawberries is a typical example. Foot rots on various graminaceous hosts are generally produced by species of *Helminthosporium*.

The mode of action of the pathogens causing root and foot rots differs from that of some of the soft rots since most of these organisms are able to degrade both pectic substances and cellulose. Hence they attack not only young seedlings but also plants that are hard and mature containing relatively less pectic material and more cellulose than the former. Although experimental data for all species are not available, it is plausible to assume on the basis of recent investigations that all *Fusaria* that cause rotting in living plants produce pectic enzymes (Singh and Wood, 1956; Winstead and Walker, 1954) and cellulase (Venkata Ram, 1956).

Species of *Helminthosporium*, such as *H. avenae* and the others that are responsible for root and foot rots of cereals, are also reported to be cellulolytic (Marsh *et al.*, 1949). Another major cause of stem rot in a wide range of host plants is *Sclerotium rolfsii*. As was pointed out previously, this organism produces both pectinolytic and cellulolytic enzymes.

Relatively few of the toxins of the root- and foot-rotting fungi have been investigated in relation to their role in tissue disintegration. In studies on *Helminthosporium sativum*, the causal agent of foot rot and

seedling blight of wheat, a toxin injurious to seedlings was obtained (Ludwig, 1957). In combination with the enzymes of the fungus, this toxin may cause cellular disintegration and death. In the crown rot disease of peanuts caused by *Aspergillus niger*, necrosis was apparently induced by oxalic acid (Gibson, 1953). Pathogenicity of isolates was correlated with ability to form oxalic acid in culture. This is one of the few instances in which such a relationship has been demonstrated. The essentiality of a toxic substance as a precursor to tissue breakdown is also exemplified in the development of winter crown rot of alfalfa. The primary factor producing necrosis was the formation of high concentrations of hydrocyanic acid (HCN) by an unidentified Basidiomycete (Lebeau and Dickson, 1955). Intra- and intercellular development of the pathogen occurred in host cells only after injury by HCN.

The relatively high lignin content of many of the monocots that are subject to foot rots suggests that decomposition of this cell wall component may play a part in the breakdown of tissue of these plants. The evidence that certain *Fusaria*, including *F. lactis* and *F. nivale* (the snow mold fungus), readily decompose lignin (Fischer, 1953) supports this assumption.

### C. Necrosis and Destruction of Foliage

The plant diseases discussed thus far have been those in which the causal organisms injure mainly through the action of cell wall dissolving enzymes alone, or through these enzymes in combination with toxic substances that are non-enzymatic in nature. In diseases of this type, rotting of the tissue is the primary disease process, and substances that cause necrosis of cells or tissues in most instances have been considered to be of secondary importance. However, in a large group of foliage diseases, the respective pathogens cause local or systemic necrosis and death of foliar tissues mainly through the action of toxins or related substances that injure and disintegrate cell protoplasm. The initial disorganization and degradation of cell wall structure is of less significance for the foliar diseases than for those diseases discussed previously.

In this category there are two main types of diseases: the leaf spots and the blights.

#### 1. Leaf Spots

When a plant pathogen forms localized lesions, consisting of dead and collapsed cells, on leaves of host plants, the symptom is referred to as a "leaf spot." In foliage infections of this type, the central localized area or holonecrotic area containing dead or dying cells is usually surrounded by a band of tissue, the plesionecrotic zone, in which cells are still alive but somewhat injured by toxic materials (Cunningham, 1928;

Akai, 1951). Presumably, these toxins may be formed directly by the pathogen or possibly to a lesser degree by the dying host cells in the center of the spot.

A unique aspect of many spot diseases is their apparent self-limiting nature and the discrete pattern of the necrotic area. Particularly in the case of fungus pathogens, disintegrating effect on cell wall constituents is much less evident than is the marked injury and degeneration of the protoplasm of the cell itself. Leaf cells in the holonecrotic zone exhibit one of two types of necrosis, involving either coagulation or dissolution of protoplast components.

In an attempt to determine whether host reactions—as manifested by morphologic changes—are responsible for localization of leaf pathogens, Cunningham (1928) made a histologic study of many different leaf spotting fungi. Included were species of *Cercospora*, *Septoria*, *Mycosphaerella*, *Phyllosticta*, and *Alternaria*. In general, cell contents disappeared in the center of the holonecrotic zone and cell walls were collapsed but not disintegrated. In the plesionecrotic zone, intercellular mycelium was often present throughout the area and frequently extended to the point where host cells were apparently quite normal. Commonly, nuclei and chloroplasts in cells of the plesionecrotic zone had either completely disappeared or remained only in outline, the contents having disappeared. Similar observations were also recorded by Akai (1951). Analysis of these effects indicates that proteolytic as well as other enzymes of these pathogens were undoubtedly playing an important role in the apparent changes in the protoplast with the hydrolytic cell-wall-destroying enzymes playing a lesser role.

Although primary effects include degradation of protoplast constituents, many of these fungi such as *Alternaria* and *Helminthosporium* can also degrade pectic and cellulosic materials *in vitro* (Winstead and Walker, 1954; Marsh *et al.*, 1949; Shigeyasu, 1951). This, however, is only an indirect indication that such enzyme systems are active in host tissues.

Cellulase formation has not been demonstrated for the bacterial pathogens capable of causing leaf spots. However, pectinolytic enzymes are formed by *Pseudomonas tabaci*, the cause of wildfire of tobacco, and *P. angularum*, the angular leaf spot bacterium (Wolf, 1923), and a large number of other bacterial leaf pathogens (Oxford, 1944; Sabet and Dowson, 1951; Smith, 1958a).

The necrogenic capabilities of the leaf spot organisms have naturally led to the conclusion that they form highly potent specific toxins. This concept is supported by the work on alternaric acid and the wildfire toxin that was briefly discussed in Section II of this chapter.

Additional reports of toxin formation by other fungi that cause leaf spots have been made, but in most cases specific modes of action have not been determined nor has proof been offered that they are also formed in tissues of invaded plants.

## 2. *Blight*s

Appropriately referred to as a blight is an attack by a fungus or a bacterium which produces on leaves, branches, twigs, and floral organs a general and extremely rapid browning resulting in death. It may result from severe infection, either by leaf-spotting, foliage-invading organisms that also rapidly attack tender portions of stems and branches, or by pathogens that specifically attack the branches or twigs and subsequently cause rapid death of leaves or floral organs.

Blight and leaf spots resemble one another in the external symptoms they produce as well as in the mechanisms of pathogenesis. However, in many blights, the attacking organism may kill the host partially or wholly, not only by direct destruction of tissue in many localized areas but also by the systemic dispersal of toxic substances far beyond the original infection zone. A discussion of systemic effects of these types, however, is beyond the scope of the present chapter.

Excluding, therefore, the effects of systemic toxins, pathogenic effects of blights include the local action of toxins on leaves or young twigs and actual dissolution of cell walls by enzymes of the pathogens involved. Examples of several of these have been discussed briefly in Section II of this chapter. Although toxins are undoubtedly the major factor in rapid blighting of plants, disintegration of cell walls is necessary to some degree if the pathogen is to grow and spread rapidly through the host. The rapid destruction of the physical barrier limiting the pathogen may also aid the parasite in obtaining nutrients from the host. Thus, at least in these cases where a rotting or disintegration of tissue occurs, enzymes may also be indirectly involved in producing blight symptoms.

Studies on the pectic or cellulolytic enzymes of most blight-inducing pathogens have been very limited in number and scope, and in most instances direct evidence for the production of these enzymes is still lacking.

## D. *Decay of Xylem Tissue*

### 1. *Wilt Diseases*

When water fails to move to the leaves of a plant through the xylem and the foliage loses turgor, the condition is referred to as wilting. The



phenomenon of pathological wilting brought about by certain bacteria and fungi that invade vascular tissue of living plants is obviously a complicated physicochemical process. The detailed treatment of wilting mechanisms will be presented in Chapter 9 of this volume, and the present discussion will be limited to those processes in a wilting plant that contribute to a breakdown of vascular tissue.

Inasmuch as all vascular pathogens, whether bacteria or fungi, are facultative parasites, they must invade and colonize the host to a certain extent before they can produce wilting. To accomplish this colonization, they require nutrients and energy, a part of which must be derived from the cell walls of the vascular tissue. Thus, the enzymes secreted by these wilt pathogens may act not only on the cell walls of vessels and tracheids but also on the reserve food in the xylem parenchyma. The resultant breakdown of tissue is so limited initially that no microscopic symptoms of actual dissolution of tissue are evident. As it becomes extensive and readily visible, it may in itself eventually lead to death and collapse of the plant. In trees and woody shrubs, little apparent damage is done to cell walls because of considerable lignification of cell walls in the vascular tissue. In oak wilt, caused by *Ceratocystis fagacearum* (Fergus and Wharton, 1957), and the Dutch elm disease, caused by *Ceratocystis ulmi*, microscopic observation alone often fails to indicate any serious enzymatic digestion of vessel walls.

Succulent herbaceous annuals often show an extreme type of tissue breakdown when attacked by bacterial wilt pathogens. In young tomato, tobacco, or potato plants invaded by the Granville wilt bacterium, *Pseudomonas solanacearum*, cells in the xylem, phloem, and adjacent pith and cortex may be rapidly disintegrated, resulting in a soft decay of localized areas or pockets in the stem (Husain and Kelman, 1958). In hardened, mature plants, with more advanced lignification than in younger plants, little tissue breakdown is evident. Similar but less severe decay of xylem tissue occurs in other bacterial wilt diseases.

Although microscopic studies reveal the degradation of pectic materials in host tissues, the actual nature of pectic enzymes formed by bacteria that invade vascular tissue has been investigated in only a few cases. The pectinolytic ability of several bacteria of this type has been reported, including *Xanthomonas campestris* (Wolf, 1923; Sabet and Dowson, 1951; Smith, 1958a, b), *Corynebacterium sepedonicum* (Oxford, 1944), and *C. michiganense* (Sabet and Dowson, 1951). In a study on the mechanisms of pathogenesis of the Granville wilt bacterium, *Pseudomonas solanacearum*, production of both PME and PG was noted (Husain and Kelman, 1958). Histological studies revealed that breakdown of pectic substances in the middle lamella and cell walls occurred

in wilted tomato and tobacco plants invaded by this pathogen. Furthermore, PG was present in host plants infected with the bacterium (Husain and Kelman, 1957), and culture filtrates containing PME and PG caused disorganization of tissue similar to that occurring in diseased plants.

In wilt diseases of herbaceous plants caused by members of the genera *Fusarium* and *Verticillium*, disorganization of xylem tissue is initially less evident than in the bacterial wilts. Tomato plants affected by *Fusarium oxysporum* f. *lycopersici* show severe breakdown of vascular tissue only in the last stages of disease and then only if the plant is young. In cotton plants with woody tissue infected by *Fusarium oxysporum* f. *vasinfectum*, microscopic symptoms of cell disintegration may not be evident; however, partial degradation of cell walls occurs even here.

Considerable information has become available in the past few years relative to ability of the vascular *Fusaria* (Gothoskar *et al.*, 1955; Waggoner and Dimond, 1955; Winstead and Walker, 1954) and similar fungi (Scheffer *et al.*, 1956; Kamal and Wood, 1956) to form specific pectic enzymes such as PME, PG, or DP *in vitro*.

In most of the work with vascular fungi, the enzymes have been studied only *in vitro* and their role in the disease process has not been conclusively demonstrated. It is probable, however, that all of the species of *Fusarium* and *Verticillium* that invade vascular tissues can break down pectic substances in their respective host plants.

Those vascular parasites that attack woody plants also produce pectic enzymes, although in smaller amounts. The Dutch elm disease fungus, *Ceratocystis ulmi*, has been shown to produce low levels of PG in culture (Beckman, 1956). Since xylem tissue of the elm contains only a very small quantity of pectic materials, the degradation of pectic materials is probably less important in causing tissue disintegration in this disease than in wilt diseases of herbaceous plants (Husain and Dimond, 1958c).

Little information is available concerning the specific role and relative importance of cellulolytic enzymes in disintegration of plant tissue by vascular parasites. The wilt-inducing *Fusaria* that have been studied can degrade cellulose to some extent (Venkata Ram, 1956). In particular, the tomato wilt pathogen, *F. oxysporum* f. *lycopersici*, is strongly cellulolytic and produces high levels of cellulase in culture (Husain and Dimond, 1958a). *Verticillium alboatrum* also produces cellulase (Edgington, L. V., personal communication). Only weak cellulase activity has been noted in culture filtrates of *Ceratocystis ulmi* (Beckman, 1956; Husain and Dimond, 1958c).

The breakdown of cellulose in plant cell walls may be brought about by enzyme systems of certain vascular bacterial pathogens, although it

has been generally assumed that bacterial plant pathogens are non-cellulolytic. A cellulase (Cx), capable of causing a rapid hydrolysis of carboxymethyl cellulose, was shown to be present in culture filtrates of *Pseudomonas solanaccarum* (Husain and Kelman, 1958). Levels of the Cx enzyme were higher in culture filtrates of a pathogenic strain than in filtrates of weakly pathogenic or non-pathogenic isolates. This Cx enzyme was also present in diseased tomato plants and it was apparently produced by the pathogen, since no Cx activity was detected in non-infected plants (Husain and Kelman, 1957). The conclusion that native cellulose was altered in the walls of invaded vascular cells was based on the observation that birefringence in polarized light was not evident in walls of cells in areas invaded by the bacterium.

The above observations indicate that cellulase may be active in diseased plants, but conclusive evidence for the exact role of cellulase in natural infections by vascular pathogens is still lacking.

## 2. Wood Decays

Approximately 2000 species of fungi belonging to the class Basidiomycetes have been categorized as wood decay fungi. Timber, shade, and ornamental trees are commonly attacked by fungi that cause disintegration of the heartwood of these trees. In terms of the total number of fungi that cause decay, only a small percentage, however, can cause decay of heartwood of living trees. The heart-rot fungi are usually unable to attack living tissues in the sapwood of invaded trees while the tree is still alive. It is strange that the decay fungi that cause heart-, butt-, or root-rots of living trees rarely cause decay of wood in service and that they may cease their activity following the death of the host tree. An entirely different succession of decay fungi usually completes the destruction of dead timber, invading first sapwood and then heartwood. A limited number of phytopathogenic Ascomycetes and Fungi Imperfecti are also capable of causing wood decay, but it is doubtful that any bacterial plant pathogens can actively decompose wood.

The extensive literature on the degradation of wood and its components by microorganisms has been reviewed by Cartwright and Findlay (1946) and more recently by Campbell (1952). The related literature on lignin decomposition has also been summarized by Brauns (1952) and Lawson and Still (1957). Thus, a detailed summary of the research contributions in this field will not be presented.

Microscopically, the structure of the cell walls in decayed wood is altered. Cell walls may show spiral cracks, small holes, or large cavities caused by dissolution of the structural materials. As has been illustrated by Bailey and Vestal (1937) the characteristic shape of cavities in cell

walls reveals the remarkable specificity of the enzymatic hydrolysis since it progresses along two planes predetermined by the chemical structure of cellulose. Often open pockets are seen where tissue has been completely dissolved. Bore holes formed by the hyphae of wood decay fungi may be smaller or several times larger than the hyphae that penetrated the wall (Proctor, 1941). Corrosion of walls, thinning, splitting of the middle lamellae, and enlargement of pit openings are other effects of decay.

These physical changes reflect profound biochemical effects resulting from the degradation of cellulose, lignin, hemicellulose, and other non-cellulosic polysaccharides in the cell walls. On the basis of the nature of decay and type of cell wall constituents destroyed, the wood rots have been classified into two main types: the "brown rots," which preferentially destroy carbohydrates leaving lignin unaffected; and the "white rots," which preferentially attack lignin with variable effect on carbohydrates.

In addition, a third type of decay has been characterized as "soft rot." In contrast to brown and white fungi the hyphae of soft rot fungi are not usually evident in cell lumens, but they are present in cylindrical cavities formed by enzymatic action within the secondary walls of wood cells.

It is now generally accepted that a great majority of brown rot fungi attack only cellulose and non-cellulosic polysaccharides and leave the lignin intact. In fact, some of the brown rot fungi, mostly species of *Lenzites*, have been utilized in the isolation of native lignin from wood (Schubert and Nord, 1957).

While the brown rot fungi belong to a fairly homogeneous group with respect to their effects on the constituents of wood, the white rots are caused by a group of organisms that vary considerably in this respect. At one time it was thought that white rot fungi attacked only lignin in wood. On the basis of numerous studies, it is now clear that wood-rotting fungi of the white rot group are a heterogeneous group of organisms with the common capability of decomposing lignin. With respect to the relative amounts of lignin and cellulose destroyed, these fungi vary in their preferential utilization of one or the other constituents. Thus, Campbell divided these fungi into three main groups on the basis of the constituents of the wood that are initially affected: (1) lignins and pentosans are attacked in early stages and attack on cellulose is delayed until later stages of the decay; (2) cellulose and associated pentosans are attacked first but lignin and pentosans not associated with cellulose are not degraded until the later stage of decay; (3) lignin and cellulose are attacked in early stages but in varying proportions.



The mode of disintegration of woody tissue by wood-decay fungi differs markedly from the various types of disintegration discussed thus far. Wood decays are distinctive in comparison with other disease processes in that the attacked tissue is already dead and surrounded by dead tissue. All the damage to xylem tissue is accomplished by the action of various enzymes secreted by the wood decay organisms and the wood is degraded without the need for toxic systems that kill living cells. These wood-destroying fungi are able, nevertheless, to form some extremely potent enzymes that can degrade complex organic molecules such as cellulose and lignin which are among the most resistant polymers synthesized by living plants. The unique difference in enzymatic capabilities between wood-decay fungi and other microorganisms lies in their ability to attack a substrate in which both cellulose and lignin are present in an intimate relationship. The cellulose in the cell walls of woody tissue is not significantly different from that of cotton fibers. However, it is significant that relatively few of the large numbers of cellulolytic microorganisms that are capable of destroying cotton textiles with ease can also attack wood and utilize the cellulose that it contains.

In addition to the inherent resistance to microbial decomposition of lignin itself, the retardation of the rate of cellulose decomposition in wood is attributed mainly to the presence of lignin, since as the lignin concentration decreases, the decomposition of cellulose increases. Furthermore, presence of lignin is associated with inhibition of decomposition of other carbohydrates, proteins, and other plant constituents.

The basis for this effect on cellulose decomposition by lignin is not fully understood. Although a chemical bonding between these materials has been suggested, there is no direct evidence that this is involved. Neither has it been shown conclusively that lignin or its decomposition products are directly toxic or inhibitory to the decay organisms. Furthermore, there is no evidence that lignin affects cellulose decomposition adversely when both are added separately to a culture medium. It is possible that the intimate manner of deposition of lignin within the anastomosing cellulosic framework of the cell wall imposes a requirement for a different type of enzyme or a distinctive combination of enzyme systems formed by only a limited number of fungi (Cowling, 1958).

However, as was pointed out previously in the discussion of cellulolytic enzymes, failure to invade and decay wood by organisms capable of destroying cotton textiles does not of necessity indicate that enzyme systems of these organisms cannot degrade cellulose in lignified tissue.

Numerous studies on the biochemistry of decay and the enzymes

formed by wood decay fungi have been reported. Significant early contributions were made by Bray and Andrews, Buller, Schmitz, and Zeller. Subsequent studies during the period from 1925 to 1945 by Bose and Sarkar, Bavendamm, Campbell, Findlay, Garren, Nutman, and others gave evidence of the multiplicity and diversity of enzymes formed by the hymenomycetous fungi involved in wood decay. Most of these investigations were based on assay methods that were not reliable for enzymatic work and that did not utilize pure substrates. Thus, they failed to give direct information as to the basic nature of the enzymes and the specific mechanisms of degradation for individual components of wood. Although the empirical nature of many of these studies must be recognized, Campbell (1952) points out the value of these studies since probable effects on wood components have been accurately predicted from enzyme tests and supported by analyses of sound and decayed wood.

It is now known that all wood-rotting fungi studied thus far can hydrolyze cellulose to form glucose. As was pointed out in the discussion of cellulose decomposition, it is not known whether this hydrolysis is accomplished by a multiple enzyme system or by a single cellulase. In studies on cellulase production by several fungi belonging to white and brown rot groups both types of organisms with a few exceptions produced  $C_1$  and  $C_x$  type enzymes (Reese and Levinson, 1952). Oddly enough, the brown rotters were different from the white rotters in that the former caused a smaller loss in tensile strength of cotton than did the latter. Partial purification of the cellulase of one brown rot fungus, *Polyporus palustris*, has been reported, but full details of the nature of this enzyme have not been published as yet (Higa *et al.*, 1956).

A positive correlation between ability to utilize native lignin and the production of certain types of polyphenol oxidase systems has been established (Gottlieb and Pelczar, 1951). Present knowledge of the mechanisms involved in breakdown of lignin by white rot fungi has been reviewed briefly in Section II. Interest in lignin-decomposing enzymes has expanded greatly as their potential utility in elucidating lignin structure has become evident (Schubert and Nord, 1957).

Wood-decay fungi also produce enzymes that break down hemicellulose and polysaccharides other than cellulose which occur in cell walls of woody tissues. It has been suggested by several investigators that non-cellulosic polysaccharides may be hydrolyzed by cellulase itself. The relative importance of the degradation of these non-cellulosic components in the disintegration of woody tissue has not been determined.

The role of pectic enzymes in the decay of wood is also ill defined. Several of the wood-destroying fungi do produce pectinase, but no recent

detailed studies on the nature of the pectic enzymes of wood-decay fungi are available. The wood of trees contains pectic substances, but the actual amount in comparison to that of cellulose is so small that decomposition of pectic substances is presumably of minor importance in the actual decay process.

#### IV. SUMMARY AND GENERAL CONCLUSIONS

##### *A. Evaluation of Main Concepts of Tissue Breakdown*

After completion of an evaluation of present knowledge of tissue breakdown as it occurs in many diverse diseases, it may be well to reconsider the terminology used in discussing the metabolic products of pathogens involved in tissue breakdown. These injurious substances were discussed under two main headings: enzymes and toxins. The concept that a toxin is any injurious substance affecting processes or components of the living protoplast was the basis for the separate treatment of this subject in the paper. It is recognized that the term "toxin" used in the broad sense can include any phytotoxic substance produced by a plant pathogenic microorganism and thus, one may properly consider enzymes in this category (Dimond and Waggoner, 1953). Nevertheless, there is some advantage in a treatise on the mechanism of tissue breakdown in distinguishing between (1) those effects that are known now to be purely enzymatic in nature, usually involving the non-living components of the tissue and (2) those effects that are less well defined, involving injury to protoplasm itself. These may well involve the action of enzymes as well as non-enzymatic substances of the pathogen that interfere with or stop essential metabolic processes of the living protoplast. The latter type of toxic material may be a product of direct synthesis by the pathogen or result from a host-parasite interaction.

The pathogen-suscept relationship in most diseases involving tissue disintegration is superficially a relatively simple type of interaction. The deceptiveness of this outward simplicity is evident when one considers the appreciable number of papers on this subject in contrast to the paucity of conclusive and direct evidence that has been presented to explain in biochemical terms one or more major processes of pathogenesis. It is unfortunately true that for almost 50 years much of the phytopathological research on tissue disintegration has involved the "shotgun approach" in which crude culture filtrates containing a multiplicity of enzymes and metabolites were applied to substrates of equally complex and diverse composition. A significant stimulus has been provided by recent advances in knowledge of both the chemical composition of the major components of plant tissues and the biochemistry of

microbial degradation of plant products. The effect of this stimulus is apparent in the increased number of papers during the last 5 years concerned with the true nature of decay processes as they relate to the science of phytopathology. It is possible, at present, to organize this information by considering the relative importance that the breakdown of certain major substrates of the host has in relation to the disintegration of the tissue as a whole.

On the basis of the nature of the host tissue, diseases causing tissue disintegration can be classified into two major groups: (1) those that affect mainly parenchymatous tissue, which is high in pectic materials, and (2) those that affect mainly tissue, in which lignification has taken place to some degree. Parenchymatous decays, which are the most numerous, characteristically involve the disintegration of more or less localized areas that are initially surrounded by masses of cells that are still alive. In this circumstance, the progressive death of cells as the lesion enlarges is a concomitant of pathogenesis whether this death precedes, accompanies, or follows degradation of cell wall components. Subdivisions within parenchymatous decays can be made on the basis of the host component that is affected mainly during the initial development of the pathogen.

In the case of certain leaf spot, anthracnose, and leaf blight diseases, the marked changes in the protoplast that occur prior to and immediately following invasion of the tissues by the pathogen constitute evidence that for diseases of this type, protoplasmic injury may be the major initial step in the disintegration process. Breakdown of pectic and cellulosic substances may follow or accompany breakdown of the protoplast. In some instances, the protoplasm is disorganized and the cell may collapse without apparent breakdown of cellulose.

In most of the soft rot, damping-off, and foot rot diseases, the main host substrate under initial attack is the pectic material of the middle lamella and cell wall proper. Apparently, it is now possible to discard the classical concept that a specific enzyme, protopectinase, breaks down protopectin in host tissues since it is possible to macerate plant tissues with purified preparations of PG from very diverse sources, including non-pathogenic organisms. Furthermore, an enzyme has not been isolated as yet that will attack only natural pectin and not soluble pectin or pectic acid. Inasmuch as the existence of a distinct protopectinase is highly questionable, maceration of tissue in pathogenesis can be considered to reflect the action of a complex of pectic enzymes.

Hydrolysis of the glycosidic linkages of pectic compounds was originally considered to depend directly on the action of PG with PME affecting the rate of hydrolysis indirectly. However, recent work indicat-



ing the diverse nature of the pectic enzymes of different microorganisms has made it necessary to revise the terminology of the PG type of enzymes. The wide pH range for activity of certain enzyme preparations and the variety of end products obtained in hydrolysis of pectic substances suggest that mixtures of different pectic enzymes have been used by many investigators. However, it is evident, on the basis of those few instances in which highly purified preparations have been tested, that several different enzymes are capable of hydrolyzing the glycosidic linkage in pectic substances. It is not possible at present to resolve the confusion that exists in this area. Furthermore, since the inherent nature of the pectic enzymes of most plant pathogens has been inadequately characterized, very few of these enzymes can be placed within the new classification devised by Demain and Phaff (1957).

There is strong evidence that certain facultative parasites such as *Botrytis cinerea* bring about tissue disintegration—including injurious effects on living cells—by purely enzymatic effects, with pectolytic action the main initial process. It seems difficult, however, to conceive that both the maceration and death of cells can be brought about by means of a single highly specific enzyme system that causes a degradation of one component of the host. However, the death of cells that accompanies the macerating effect is so closely associated with the enzymatic degradation of pectin that the presence or absence of a separate protoplasmic toxin will only be resolved conclusively by the use of highly purified enzyme preparations. It can be postulated that degradation of the middle lamella alone need not cause death of cells although toxic effects may be intensified by this process. This viewpoint is supported by evidence that plant tissue can be macerated with purified pectic enzyme preparations of certain non-pathogenic fungi without bringing about rapid death of cells. Another unique situation in which pectic materials are broken down in living tissue without bringing about death of cells is the invasion of the roots of certain tree species by the ectotrophic type of mycorrhizal fungi. The intercellular penetration of tree roots by hyphae of these fungi is accompanied by a dissolution of middle lamellae without apparent injury to the host.

Certain fungi combine a relatively potent cellulolytic enzyme system with pectic enzymes to make a two-pronged attack on the framework of the cell. The ability to break through and decompose the cellulosic barriers of the cell walls provides a mechanism for the rapid spread of such rotting fungi. In general, soft rot bacteria and many phycomycetous fungi which either lack cellulolytic enzymes or do not actively degrade cellulose are usually less effective than fungi such as *Sclerotium rolfsii* or

*Rhizoctonia solani* in their invasion and disintegration of tissue in which the percentage of pectic materials is relatively low.

The wilt fungi such as the *Fusaria* that invade vascular tissue of herbaceous plants are also characterized by the formation of enzyme systems capable of degrading both pectic and cellulosic substrates with equal facility. Certain of the *Fusaria* may also affect lignin as well. Although the relationship of these enzymes to the mechanism of wilting has not been clearly established, their importance in the disintegration process is unquestioned. In the case of the oak wilt fungus and the Dutch elm disease fungus, both of which affect highly lignified tissue, breakdown of xylem tissue is not extensive. Although both organisms form the pectic enzymes, the action of pectic enzymes on the highly lignified cells of the wood is obviously of little apparent functional value.

The nature of enzymatic degradation of cellulose is now under intensive investigation by workers in many diverse fields with interests ranging from fabric deterioration to rumen physiology. As in the case of the work with pectic enzymes, it is now apparent that modification is necessary in the concept that all cellulolytic organisms form one cellulase that can break down cellulose to glucose. So little work has been done on cellulolytic enzymes of plant pathogens that it is not possible to relate these findings to the much more extensive information on enzyme systems of microorganisms causing degradation of cotton fabrics. Presumably, plant pathogens will exhibit the same diversity in the nature of cellulolytic enzyme systems that is now becoming apparent for saprophytic fungi and bacteria.

The decay of lignified woody tissue is restricted mainly to a rather specialized group of fungi. If one were to rate microorganisms on an ascending scale on the basis of their ability to degrade the components of the host with relatively high resistance to decomposition, the white rot fungi might well be placed at the top because of the ease with which many of these organisms can destroy lignin. The recent studies on the nature of the polyphenoloxidase systems of certain white rot fungi will be helpful not only in understanding the mechanism of degradation of lignin but may also aid in the determination of the exact chemical structure of lignin.

#### B. Needs for Future Research on the Mechanisms of Tissue Disintegration

In attempting to digest available information on the mechanisms of tissue breakdown by microorganisms, one is naturally impressed by certain obvious gaps in present knowledge. In addition to the absence

of knowledge of specific properties of pectic enzymes, cellulolytic enzymes, and polyphenoloxidase systems of most plant pathogens, the role of enzymes that affect other major components such as the hemicelluloses and related non-cellulosic carbohydrates is almost completely unknown. The pressing need for the use of relatively pure enzyme preparations in future studies is apparent, although the difficulty of obtaining such preparations is admittedly very great.

In most instances, toxins affecting living cells of plants have been shown to be non-enzymatic in nature (Brian, 1955). However, in the case of bacterial toxins formed by animal and human pathogens, many of these substances are enzymatic and certain of them injure their hosts by attacking proteinaceous substrates (Oakley, 1954). These observations on animal toxins suggest that the possible role of proteolytic enzymes in tissue disintegration by plant pathogens should not be overlooked. The marked changes in permeability of the plasma membranes of cells surrounding an enlarging zone of necrotic tissue may well indicate changes in these membranes induced by enzymatic action on membrane surfaces. The association of lipids and proteins in the formation of the plasma membrane of living cells suggests that lipolytic as well as proteolytic enzymes should also be considered in this connection.

Within any living cell there exists the potential for the formation of enzymes capable of bringing about destruction of the cell components by a reversal of the original mechanism that created these compounds. In the evaluation of pathogenesis, the part which such enzymes might play in tissue disintegration has not been determined.

The recent progress that has been made in advancing knowledge of the mechanisms of tissue breakdown serves to emphasize the pressing need for additional information on the basic nature of the enzymes involved in degradation of the main structural components of the cell wall as well as the manner in which living protoplasts are killed and decomposed. Knowledge of this type is fundamental and essential in elucidating the mechanism of pathogenesis which is one primary objective of phytopathology as a science. Furthermore, this information may serve as the basis for new and effective methods for the prevention of tissue disintegration in living plants or plant products, a problem of major economic importance.

#### REFERENCES

- Adler, E. 1957. Structural elements of lignin. *Ind. Eng. Chem.* **49**: 1377-1383.  
Aitken, R. A., B. P. Eddy, M. Ingram, and C. Weurman. 1956. The action of culture filtrates of the fungus *Myrothecium verrucaria* on  $\beta$ -glucosans. *Biochem. J.* **64**: 63-70.

- Akai, S. 1951. On the anatomy of necrotic lesions on leaves and stems of plants affected by pathogenic fungi. *Mem. Coll. Agr. Kyoto Univ.* **61**: 30 pp.
- Ammann, A. 1951. Über die Bildung von Zellulase bei pathogenen Mikroorganismen. *Phytopathol. Z.* **18**: 416-446.
- Ashcroft, J. M. 1934. European canker of black walnut and other trees. *West Va. Agr. Expt. Sta. Bull.* **261**: 1-52.
- Ashour, W. E. 1954. Pectinase production by *Botrytis cinerea* and *Pythium debaryanum*. *Brit. Mycol. Soc. Trans.* **37**: 343-352.
- Bachmann, E. 1956. Der Einfluss von Fusarinsäure auf die Wasserpermeabilität von pflanzlichen Protoplasten. *Phytopathol. Z.* **27**: 255-288.
- Bailey, I. W., and M. R. Vestal. 1937. The significance of certain wood-destroying fungi in the study of the enzymatic hydrolysis of cellulose. *J. Arnold Arboretum (Harvard Univ.)* **18**: 196-205.
- Beckman, C. H. 1956. Production of pectinase, cellulases and growth-promoting substance by *Ceratostomella ulmi*. *Phytopathology* **46**: 605-609.
- Bishop, C. T., and D. R. Whitaker. 1955. Mixed arabinose-xylose oligosaccharides from wheat-straw xylan. *Chem. & Ind. (London)*. Pt. I (5): 119.
- Bramble, W. C. 1936. Reaction of chestnut bark to invasion by *Endothia parasitica*. *Am. J. Botany* **23**: 89-94.
- Bramble, W. C. 1938. Effect of *Endothia parasitica* on conduction. *Am. J. Botany* **25**: 61-65.
- Brandenburg, E. 1950. Über die Bildung von Toxinen in der Gattung *Pythium* und ihre Wirkung auf die Pflanzen. *Nachrbl. deut. Pflanzenschutzdienst (Braunschw.)* **2**: 69-70.
- Braun, A. C. 1955. A study on the mode of action of wildfire toxin. *Phytopathology* **45**: 659-664.
- Brauns, F. E. 1952. "The Chemistry of Lignin." Academic Press, New York. 808 pp.
- Brian, P. W. 1952. The phytotoxic property of alternaric acid in relation to the etiology of plant diseases caused by *Alternaria solani* (Ell. & Martin) Jones & Grout. *Ann. Appl. Biol.* **39**: 308-321.
- Brian, P. W. 1955. Role of toxins in the etiology of plant diseases caused by fungi and bacteria. *Symposium Soc. Gen. Microbiol.* **5**: 294-319.
- Brown, W. 1915. Studies in the physiology of parasitism. I. The action of *Botrytis cinerea*. *Ann. Botany (London)* **29**: 313-348.
- Brown, W. 1917. Studies in the physiology of parasitism. IV. On the distribution of cytase in cultures of *Botrytis cinerea*. *Ann. Botany (London)* **31**: 489-498.
- Brown, W. 1936. The physiology of host parasite relations. *Botan. Rev.* **2**: 236-281.
- Brown, W. 1955. On the physiology of parasitism in plants. *Ann. Appl. Biol.* **43**: 325-341.
- Büdiger, W. 1952. Zur pathologischen Anatomie des Leins, *Linum usitatissimum* L. *Phytopathol. Z.* **19**: 34-47.
- Campbell, W. G. 1952. The biological decomposition of wood. In "Wood Chemistry" (L. E. Wise and E. C. Jahn, eds.), 2nd ed. Reinhold, New York, pp. 1061-1116.
- Cartwright, K. St. G., and W. P. K. Findlay. 1946. "Decay of Timber and Its Prevention." Chemical Publ., New York 294 pp.
- Cole, J. S. 1956. Studies in the physiology of parasitism. XX. The pathogenicity of *Botrytis cinerea*, *Sclerotinia fructigena*, and *Sclerotinia luxa* with special reference to the part played by pectolytic enzymes. *Ann. Botany (London)* **20**: 15-38.
- Cowling, E. B. 1958. A review of the literature on the enzymatic degradation of



- cellulose and wood, Forest Prod. Lab., Forest Serv., U. S. Dept. Agr. Rept. No. 2116. 26 p.
- Cunningham, H. S. 1928. A study of the histological changes produced in leaves by certain leaf-spotting fungi. *Phytopathology* **18**: 717-751.
- Damle, V. P. 1952. Enzymic study of certain parasitic fungi. *J. Indian Botan. Soc.* **31**: 13-55.
- De Bary, A. 1886. Über einige Sclerotinien und Sclerotienkrankheiten. *Botan. Z.* **44**: 377-474.
- Demain, A. L., and H. J. Phaff. 1957. Recent advances in the enzymatic hydrolysis of pectic substances. *Wallerstein Labs. Commun.* **20**: 119-140.
- Deuel, H., and E. Stutz. 1958. Pectic substances and pectic enzymes. *Adv. in Enzymol.* **20**: 341-382.
- Dillingham, E. O. 1955. Cellulolytic activity and growth characteristics of *Pythium irregulare*. *Dissertation Abstr.* **15**: 678-679.
- Dimond, A. E., and P. E. Waggoner. 1953. On the nature and role of vivotoxins in plant disease. *Phytopathology* **43**: 229-235.
- Echandi, E., and J. C. Walker. 1957. Pectolytic enzymes produced by *Sclerotinia sclerotiorum*. *Phytopathology* **47**: 303-306.
- Echandi, E., S. D. Van Gundy, and J. C. Walker. 1957. Pectolytic enzymes secreted by soft rot bacteria. *Phytopathology* **47**: 549-552.
- Elliott, C. 1951. "Manual of Bacterial Plant Pathogens," 2nd ed. Chronica Botanica, Waltham, Massachusetts. 186 pp.
- Erdtman, H. 1957. Outstanding problems in lignin chemistry. *Ind. Eng. Chem.* **49**: 1385-1386.
- Etchells, J. L., T. A. Bell, R. J. Monroe, P. M. Masley, and A. L. Demain. 1958. Populations and softening enzyme activity of filamentous fungi on flowers, ovaries and fruit of pickling cucumbers. *Appl. Microbiol.* **6**: 427-440.
- Fabricius, J. C. 1774. Attempt at a dissertation on the disease of plants. (Translated by Margaret K. Ravn in 1926. *Phytopathol. Classics* **1**: 66 pp.)
- Fähræus, G., and G. Lindeberg. 1953. Influence of tyrosine and some other substances on the laccase formation in *Polyporus* species. *Physiol. Plantarum* **6**: 150-158.
- Fergus, C. L., and D. C. Wharton. 1957. Oak wilt. Histological studies of host reaction and pathogen. *Penna. State Univ. Agr. Expt. Sta. Progr. Rept.* **168**: 6 pp.
- Fischer, G. 1953. Biological decomposition of lignin by microorganisms. *Arch. Mikrobiol.* **18**: 291-297.
- Freudenberg, K. 1957. Structure and formation of lignin. *Ind. Eng. Chem.* **49**: 1384.
- Frey-Wyssling, A. 1953. "Submicroscopic Morphology of Protoplasm and Its Derivatives." Elsevier, New York. 411 pp.
- Fushtey, S. G. 1957. Studies on the physiology of parasitism. XXIV. Further experiments on the killing of plant cells by fungal and bacterial extracts. *Ann. Botany (London)* **21**: 273-286.
- Gäumann, E. 1956. Fusaric acid as a wilt toxin. *Phytopathology* **47**: 342-357.
- Gäumann, E., S. Naef-Roth, P. Reusser, and A. Ammann. 1952. Über den Einfluss einiger Welktoxine und Antibiotica auf die osmotischen Eigenschaften pflanzlicher Zellen. *Phytopathol. Z.* **19**: 160-220.
- Gibson, I. A. S. 1953. Crown rot, a seedling disease of groundnuts caused by *Aspergillus niger*. *Brit. Mycol. Soc. Trans.* **36**: 198-209.
- Gilligan, W., and E. T. Reese. 1954. Evidence for multiple components in microbial cellulases. *Can. J. Microbiol.* **1**: 90-107.

- Gothoskar, S. S., R. P. Scheffer, J. C. Walker, and M. A. Stahmann. 1955. The role of enzymes in the development of *Fusarium* wilt of tomato. *Phytopathology* **45**: 381-387.
- Gottlieb, S., and M. J. Pelczar. 1951. Microbiological aspects of lignin degradation. *Bacteriol. Rev.* **15**: 55-76.
- Gupta, S. C. 1956. Studies in the physiology of parasitism. XXII. The production of pectolytic enzymes by *Pythium debaryanum* Hesse. *Ann. Botany (London)* **20**: 179-190.
- Halliwell, G. 1957. Cellulolytic preparations from microorganisms of the rumen and from *Myrothecium verrucaria*. *J. Gen. Microbiol.* **17**: 166-183.
- Harter, L. L., and J. L. Weimer. 1921. Studies in the physiology of parasitism with special reference to the secretion of pectinase by *Rhizopus tritici*. *J. Agr. Research* **21**: 609-625.
- Harter, L. L., and J. L. Weimer. 1923. The relation of the enzyme pectinase to infection of sweet potatoes by *Rhizopus*. *Am. J. Botany* **10**: 245-258.
- Heald, F. D., and R. A. Studhalter. 1914. The Strumella disease of oak and chestnut trees. *Penna. Dept. Forestry Bull.* **10**: 1-15.
- Higa, H. A., R. D. O'Neill, and M. W. Jennison. 1956. Partial purification of a cellulase from a wood-rotting Basidiomycete. *J. Bacteriol.* **71**: 382.
- Higgins, B. B. 1927. Physiology and parasitism of *Sclerotium rolfsii* Sacc. *Phytopathology* **17**: 417-448.
- Higuchi, T., I. Kawamura, and I. Hayashi. 1956. Biochemical study of wood-rotting fungi. V. The enzymatic oxidation of lignin. *J. Japan Wood Research Soc.* **2**: 31-35.
- Husain, A. 1957. Production of a cellulolytic enzyme by *Sclerotium rolfsii*. (Abstr.) *Phytopathology* **47**: 17-18.
- Husain, A., and A. E. Dimond. 1958a. Production of cellulase by *Fusarium oxysporum* f. *lycopersici*. (Abstr.) *Phytopathology* **48**: 263.
- Husain, A., and A. E. Dimond. 1958b. Cellulolytic activity of some apple rotting fungi. (Abstr.) *Phytopathology* **48**: 263.
- Husain, A., and A. E. Dimond. 1958c. The function of extracellular enzymes of Dutch elm disease pathogen. *Proc. Natl. Acad. Sci. U. S.* **44**: 594-601.
- Husain, A., and A. Kelman. 1957. Presence of pectic and cellulolytic enzymes in tomato plants infected by *Pseudomonas solanacearum*. *Phytopathology* **47**: 111-112.
- Husain, A., and A. Kelman. 1958. Role of pectic and cellulolytic enzymes in pathogenesis of *Pseudomonas solanacearum*. *Phytopathology* **48**: 377-385.
- Husain, A., and A. Kelman. 1959. Pectic and cellulolytic enzymes of *Erwinia maydis*. (In preparation.)
- Husain, A., and S. Rich. 1958. Extracellular pectic and cellulolytic enzymes of *Cladosporium cucumerinum*. *Phytopathology* **48**: 316-320.
- Jones, L. R. 1905. The cytolytic enzymes produced by *Bacillus carotovorus* and certain other soft rot bacteria. *Zentr. Bakteriell., Parasitenk. Abt. II.* **14**: 257-272.
- Jones, L. R. 1909. Pectinase, the cytolytic enzyme produced by *Bacillus carotovorus* and other soft rot organisms. *Vermont Univ. Agr. Expt. Sta. Tech. Bull.* **147**.
- Kamal, M., and R. K. S. Wood. 1956. Pectic enzymes secreted by *Verticillium dahliae* and their role in the development of the wilt disease of cotton. *Ann. Appl. Biol.* **44**: 322-340.
- Kertesz, Z. I. 1951. "The Pectic Substances." Interscience, New York. 628 pp.
- Köhlmeier, J. 1956. Über den Cellulose Abbau durch einige phytopathogene Pilze. *Phytopathol. Z.* **27**: 147-182.

- Kraght, A. J., and M. P. Starr. 1953. Pectic enzymes of *Erwinia carotovora*. *Arch. Biochem.* **42**: 271-277.
- Lawson, L. R., Jr., and C. N. Still. 1957. The biological decomposition of lignin—literature survey. *Tappi* **40**: 58A-80A.
- Leach, J. G. 1923. The parasitism of *Colletotrichum lindemuthianum*. Minn. Univ. Agr. Expt. Sta. Tech. Bull. **14**, 39 pp.
- Lebeau, J. B., and J. G. Dickson. 1955. Physiology and nature of disease development in winter crown rot of alfalfa. *Phytopathology* **45**: 667-673.
- Lineweaver, H., and E. F. Jansen. 1951. Pectic enzymes. *Advances in Enzymol.* **11**: 267-295.
- Ludwig, R. A. 1957. Toxin production by *Helminthosporium sativum* P. K. and B. and its significance in disease development. *Can. J. Botany* **35**: 291-303.
- Marsh, P. B., K. Bollenbacher, M. L. Butler, and K. B. Raper. 1949. The fungi concerned in fibre deterioration. II. Their ability to decompose cellulose. *Textile Research J.* **19**: 462-484.
- Marsh, P. B., G. V. Merola, and M. E. Simpson. 1953. Experiments with an alkali swelling centrifuge test applied to cotton fiber. *Textile Research J.* **23**: 831-841.
- Matsumoto, T. 1923. Further studies on the physiology of *Rhizoctonia solani* Kühn. *Bull. Imp. Coll. Agr. Forestry (Morioka)* **5**: 1-13.
- McColloch, R. J., and Z. I. Kertesz. 1948. An unusual heat-resistant pectolytic factor from tomato. *Arch. Biochem.* **17**: 197-199.
- McCready, R. M., and H. S. Owens. 1954. Pectin, a product of citrus waste. *Econ. Botany* **8**: 29-47.
- Mehrotra, B. S. 1949. Physiological studies of the genus *Phytophthora*. I. Enzyme action. *J. Indian Botan. Soc.* **28**: 108-124.
- Menon, K. P. V. 1934. Studies in the physiology of parasitism. XIV. Comparison of enzymic extracts obtained from various parasitic fungi. *Ann. Botany (London)* **48**: 187-210.
- Miller, G. L., and R. Blum. 1956. Resolution of fungal cellulose by zone electrophoresis. *J. Biol. Chem.* **218**: 131-137.
- Norkrans, B. 1957a. Studies of  $\beta$ -glucosidase and cellulose splitting enzymes from *Polyporus annosus* Fr. *Physiol. Plantarum* **10**: 198-214.
- Norkrans, B. 1957b. Studies of  $\beta$ -glucosidase and cellulose splitting enzymes from different strains of *Collybia velutipes*. *Physiol. Plantarum* **10**: 454-466.
- Oakley, C. L. 1954. Bacterial toxins. *Ann. Rev. Microbiol.* **8**: 411-428.
- Overell, B. T. 1952. A toxin in culture filtrates of *Sclerotinia sclerotiorum*. *Australian J. Sci.* **14**: 197-198.
- Oxford, A. E. 1944. Production of a soluble pectinase in a simple medium by certain plant pathogenic bacteria belonging to the genus *Pseudomonas*. *Nature* **154**: 271-272.
- Pierson, C. F., and J. C. Walker. 1954. Relation of *Cladosporium cucumerinum* to susceptible and resistant cucumber tissue. *Phytopathology* **44**: 459-465.
- Potter, M. C. 1902. On the parasitism of *Pseudomonas destructans*. *Proc. Roy. Soc.* **70**: 392-397.
- Pound, G. S., and M. A. Stahmann. 1951. The production of a toxic material by *Alternaria solani* and its relation to the early blight disease of tomato. *Phytopathology* **41**: 1104-1114.
- Preston, R. D. 1952. "The Molecular Architecture of Plant Cell Walls." Chapman and Hall, London. 211 pp.
- Pringle, R. B., and A. C. Braun. 1957. The isolation of the toxin of *Helminthosporium victoriae*. *Phytopathology* **47**: 369-371.

- Proctor, P. 1941. Penetration of the walls of wood cells by the hyphae of wood-destroying fungi. *Yale Univ. School Forestry Bull.* **47**: 1-31.
- Ragheb, H. S., and F. W. Fabian. 1955. Growth and pectolytic activity of some tomato molds at different pH levels. *Food Research* **20**: 614-625.
- Reese, E. T. 1956. A microbiological process report—enzymatic hydrolysis of cellulose. *Appl. Microbiol.* **4**: 39-45.
- Reese, E. T., and H. S. Levinson. 1952. A comparative study of breakdown of cellulose by microorganisms. *Physiol. Plantarum* **5**: 345-366.
- Reese, E. T., and W. Gilligan. 1954. The swelling factor in cellulose hydrolysis. *Textile Research J.* **24**: 663-669.
- Reese, E. T., R. G. H. Siu, and H. S. Levinson. 1950. The biological degradation of soluble cellulose derivatives and its relationship to the mechanism of cellulose hydrolysis. *J. Bacteriol.* **59**: 485-497.
- Rönnebeck, W. 1956. Ein phytotoxisches Prinzip aus *Phytophthora infestans* de By. *Z. Pflanzenkrankh. u. Pflanzenschutz* **63**: 385-389.
- Sabet, K. A., and W. J. Dowson. 1951. Action of phytopathogenic bacteria on pectate gel. *Nature* **168**: 605.
- Scheffer, R. P., S. S. Gothoskar, C. F. Pierson, and R. P. Collins. 1956. Physiological aspects of *Verticillium* wilt. *Phytopathology* **46**: 83-87.
- Schubert, E. 1954. Einfluss von Wasserstoff- und Alkali-Ionen auf den enzymatischen Abbau von Pektin verschiedenen Veresterungsgrades durch Pektinlykosidasen und Pektinlykosidasegemische. *Helv. Chim. Acta* **37**: 691-700.
- Schubert, W. J., and F. F. Nord. 1957. Lignification. *Adv. Enzymol.* **18**: 349-378.
- Seegmiller, C. G., and E. F. Jansen. 1952. Polymethylgalacturonase, an enzyme causing the glycosidic hydrolysis of esterified pectic substances. *J. Biol. Chem.* **195**: 327-336.
- Shigeyasu, A. 1951. Cellulase of rice brown spot fungus, *Helminthosporium oryzae*. *Ann. Phytopathol. Soc. Japan.* **14**: 97-105.
- Singh, R. K., and R. K. S. Wood. 1956. Studies in the physiology of parasitism XXI. The production and properties of pectic enzymes secreted by *Fusarium moniliforme* Sheldon. *Ann. Botany (London)* **20**: 89-103.
- Siu, R. G. H. 1951. "Microbial Decomposition of Cellulose with Special Reference to Cotton Textiles." Reinhold, New York. 531 pp.
- Siu, R. G. H. 1954. Problems and speculations on the decomposition of cellulose by fungi. *Trans. N. Y. Acad. Sci.* [2] **17**: 37-44.
- Siu, R. G. H., and E. T. Reese. 1953. Decomposition of cellulose by microorganisms. *Botan. Rev.* **19**: 377-416.
- Smith, R. E. 1902. The parasitism of *Botrytis cinerea*. *Botan. Gaz.* **33**: 421-436.
- Smith, W. K. 1958a. A survey of the production of pectic enzymes by plant pathogenic and other bacteria. *J. Gen. Microbiol.* **18**: 33-41.
- Smith, W. K. 1958b. Chromatographic examination of the products of digestion of pectic materials by culture solutions of plant pathogenic and other bacteria. *J. Gen. Microbiol.* **18**: 42-47.
- Tamari, K., and J. Kaji. 1955. On the biochemical studies of the blast mold (*Piricularia oryzae* Cavara), the causative mold of the blast disease of the rice plant. Part 2. Studies on the physiological action of piricularin, a toxin produced by the blast mold, on rice plants. *J. Agr. Chem. Soc. Japan.* **29**: 185-190. (Japanese with English summary.)
- Tribe, H. T. 1955. Studies in the physiology of parasitism. XIX. On the killing of plant cells by enzymes from *Botrytis cinerea* and *Bacterium aroideae*. *Ann. Botany (London)* **19**: 351-368.



- Van-Hall, C. J. J. 1903. Das Faulen der jungen Schösslinge und Rhizome von *Iris florentina* und *Iris germanica*, verursacht durch *Bacillus omnivorus* v. Hall und durch einige andere Bakterienarten. *Z. Pflanzenkrankh. u. Pflanzenschutz* **13**: 129-144.
- Venkata Ram, C. S. 1956. Studies on cellulolytic activity of *Fusaria* with reference to bacterial and other cellulose substrates. *Proc. Natl. Inst. Sci. India* **22**: 204-211.
- Waggoner, P. E., and A. E. Dimond. 1955. Production and role of extracellular pectic enzymes of *Fusarium oxysporum*, f. *lycopersici*. *Phytopathology* **45**: 79-87.
- Ward, H. M. 1888. A lily-disease. *Ann. Botan.* **2**: 319-382.
- Whistler, R. L., and C. L. Smart. 1953. "Polysaccharide Chemistry." Academic Press, New York. 493 pp.
- Whitaker, D. R. 1953. Purification of *Myrothecium verrucaria* cellulase. *Arch. Biochem. Biophys.* **43**: 253-268.
- Whitaker, D. R. 1957. The mechanism of degradation of cellulose by *Myrothecium verrucaria* cellulase. *Can. J. Biochem. and Physiol.* **35**: 733-742.
- Winstead, N. N., and J. C. Walker. 1954. Production of vascular browning by metabolites from several pathogens. *Phytopathology* **44**: 153-158.
- Wolf, F. A. 1923. Studies on the physiology of plant pathogenic bacteria. VII. Pectic fermentation in culture media containing pectin. *Phytopathology* **13**: 381-384.
- Wolf, F. A., and J. M. Flowers. 1957. Tobacco anthracnose and toxin production. *Tobacco Sci.* **1**: 93-98.
- Wood, R. K. S. 1955a. Studies in the physiology of parasitism. XVIII. Pectic enzymes secreted by *Bacterium aroideae*. *Ann. Botany (London)* **19**: 1-27.
- Wood, R. K. S. 1955b. Pectic enzymes secreted by pathogens and their role in plant infection. *Symposium Soc. Gen. Microbiol.* **5**: 263-293.
- Wood, R. K. S., and S. C. Gupta. 1958. Studies in the physiology of parasitism. XXV. Some properties of the pectic enzymes secreted by *Pythium debaryanum*. *Ann. Botany (London)* **22**: 309-320.

## CHAPTER 6

# Growth Is Affected

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## I. INTRODUCTION

One of the most striking characteristics of higher plants and animals is the extraordinary way in which all of their functional parts fall into a coherent and flexible, but definitely limited pattern. In the normal organ-

ism, morphogenetic laws are strictly obeyed and processes concerned in metabolism, growth, cellular differentiation, and organogenesis are precisely regulated. These processes start and stop in harmony to give the organism certain proportions that remain constant from one generation to the next.

The normal plant may be looked upon as a precisely balanced complex of interrelated reactions that are in a state of dynamic equilibrium. Any deviation in this complex of reactions will tend to disrupt the balanced system and may lead to atypical, abnormal, or pathological growth. It is not surprising, therefore, that growth patterns of plants may be readily modified by external environmental conditions such as heat, cold, light, and humidity, by nutritional deficiencies and excesses, by radiation, by changes in genetic constitution, as well as by pathogenic organisms of the most diverse types. The borderline between the normal and the abnormal in plants is, therefore, often quite indistinct and may represent merely quantitative changes which are evidenced either by a harmonious but somewhat exaggerated manifestation of the normal developmental processes or, as is more commonly found to be true, by an arrest or inhibition of the processes concerned with growth and development. At other times, orderly qualitative changes result which in their most interesting form reveal growth and developmental potentialities in a plant far beyond any realized in the past history of the species. In the most extreme instances, plant cells undergo far-reaching qualitative changes and may, as a result, become permanently modified into new cell types in which continued unregulated and unorganized growth, rather than differentiation, characterizes the behavior of the affected cell.

Of particular concern to the present discussion is the influence that parasites of many different kinds exert on the growth and development of a parasitized plant.

Gäumann (1954) has indicated that organisms are pathogenic only if they are toxigenic. Although this sweeping generalization probably contains a large element of truth, little is actually known yet about the physiological and chemical mechanisms underlying disease. It is nevertheless true that the growth of systemically diseased plants is generally inhibited. Even in those instances in which the disease is very mild and symptoms are largely masked (latent virus infections of potato, masked strains of tobacco mosaic virus), a statistically significant inhibition of growth of the host plant is found to occur. More often, pronounced stunting is a characteristic manifestation of disease in plants. This may result either from systemic infection, or from the diffusion of specific chemical compounds elaborated by a pathogen, which itself remains localized in

the host. These chemical substances fall essentially into two categories: (1) enzymes; (2) soluble metabolites, many of which are capable of reproducing perfectly the toxic manifestations of disease and some of which have been characterized chemically. (See further discussion in Chapter 13 (Volume II of this treatise).

Of far greater biological interest than the substances which act in such an unsophisticated way to damage, kill, or otherwise inhibit growth of the cells of the host, are those substances that stimulate cells to excessive growth.

The regulation of growth, differentiation, and organ formation in higher plants appears to result, among other things, from a very precisely balanced series of growth-regulating substances or hormones, on the one hand, and from inhibitory systems or other compensatory mechanisms that control the synthesis and response of plant cells to such biologically active substances, on the other. Certain metabolites elaborated by pathogens appear to be quite similar or identical in their physiological action to growth-regulating hormones found to occur naturally in a plant. When these are produced in excess by the pathogen during the course of infection, growth responses occur—in a host—that simulate those found following the artificial application of excessive amounts of such growth-promoting substances. At other times, the chemical stimuli produced by the pathogen are quite different from any thus far recognized in the normal plant and, being different, tend to divert the normal growth pattern of the plant into new and unusual directions. Such morphogenetic stimuli appear to be of many different types. They are commonly highly specific in their action and regularly induce unusual growth responses in an affected plant. It must be recognized, however, that whatever form the growth abnormality takes, the potentialities for this form must have been present in the cells of the host. These growth potentialities are simply called into activity by the inciting stimulus. Thus, the morphogenetic stimulus produced by the pathogen as well as the protoplasmic substrate of the host upon which the stimulus acts are critical in determining the type of growth that results.

Finally, the metabolite elaborated by the pathogen may not itself be a hormone or a morphogenetic stimulus of the type referred to above which directly influences the growth pattern of the host. It may instead affect specifically the regulatory mechanism of the host cells by eliminating either temporarily or permanently those cellular systems that are concerned with the regulation of growth or, alternatively, it may activate within the affected cell growth-substance-synthesizing systems whose



products are concerned specifically with growth and developmental processes. In these instances, it is the affected cell itself that elaborates growth-promoting substances—in greater than regulatory amounts—in response to a specific stimulus transmitted by a pathogen.

## II. HARMONIOUS CHANGES INVOLVING EXAGGERATED GROWTH RESPONSES

### A. Generalized Stimulation

#### 1. *Bakanae* Effect

Intensified manifestations of normal developmental potentialities have been recorded in a number of plant species following infection with a variety of fungi, many of which are obligate parasites. The common houseleek, *Sempervivum hirtum*, grows as a rosette. The leaves of this plant are broadly ovate in form and are about twice as long as they are broad. Following infection with the rust *Endophyllum sempervivi* the appearance of the leaves is altered. They grow strongly in length and may be seven times as long as they are broad. As a result, the leaves assume a linear shape. Infected leaves stand erect and are much paler in color than are their normal counterparts.

Elongation of the internodes with a resulting significant increase in size appears to be a characteristic response of certain plants to infection by specific pathogenic fungi. Following infection of one of the spurges, *Euphorbia cyparissias*, by the aecial stage of the rust *Uromyces pisi*, the stem of the host elongates greatly. The distances between successive leaves in healthy *Euphorbia* plants are about 0.5 mm. while those of infected plants are 2–3 mm. The foliage leaves of the normal plant are thin, flexible, and about twelve times as long as they are broad. In diseased specimens, the leaves are thick, brittle, and only about two to three times as long as they are broad. Infected plants have an etiolated appearance. Similar responses have been observed in *Vaccinium vitis-idaea* infected with the teleutospore stage of *Melampsora goeppertiana*, in sugar cane with the downy mildew *Sclerospora sacchari*, and in *Bromus erectus* with the smut fungus *Ustilago hypodytes*. Alterations produced on the shoots of the periwinkles *Vinca herbacea*, *Vinca major*, and *Vinca minor* by the uredospore stage of the rust *Puccinia vincae* and on the shoots of *Cirsium arvense* by the teleutospore stage of *Puccinia suaveolens* are also very similar to those described above for *Euphorbia*. The stems of the infected plants become much elongated, while the leaves are shorter, broader, yellow in color, and brittle when compared with the normal. Frequently, the shoots blossom prematurely and the flowers are more or less abortive. When, for example, *Primula clusiana* and *P. minima* are infected with *Uromyces primulae integri-*

*foleae*, not only do the rosette leaves elongate but the flowers open in the autumn of the same year rather than in the following spring as happens in the normal uninfected specimens.

Pilet (1952, 1953) studied the auxin content of healthy leaves of *Sempervivum* sp. infected with *Endophyllum sempervivi* as well as of healthy and diseased leaves of *Euphorbia cyparissias* infected with *Uromyces pisi* in an attempt to account—on a physiological level—for elongation of the stem as a result of infection. Parasitized leaves of both plant species revealed a much higher auxin content than normal. On the basis of these observations, Pilet (1953) suggested (1) that the causal fungus elaborates auxins, (2) that the increased auxin level found in the host is produced by the host in response to the parasite, or (3) that the parasite produces something which activates precursors of auxins in the host cell and which in turn are converted into auxin. It has not yet been shown, however, that the application of auxin to normal plants results in an elongation of leaves and internodes comparable to that obtained after infection by specific rust fungi. Brian (1957) has, therefore, suggested that the increased auxin found in the leaves and shoots of such plants may be coincidental rather than causal since quite different metabolic products of fungi, the gibberellins, have been shown to be responsible for stem elongation in the bakanae disease of rice as well as in many other plant species when these substances are applied artificially.

The bakanae or foolish seedling, disease of rice is the best understood of this type of disease. It has been studied by Japanese pathologists for many years. A comprehensive review of this disease has recently been presented by Stowe and Yamaki (1957). The bakanae disease, which is caused by *Fusarium moniliforme* (imperfect stage) or *Gibberella fujikuroi* (perfect stage), is widely distributed and found in most rice-growing regions of the world. *Fusarium moniliforme* has a large host range, is soil-borne, and attacks the roots and basal portions of the stem not only of rice plants but of maize, cotton, sugar cane, and other plant species as well. In the case of rice, a browning of the tissue usually occurs at the site of infection, the leaves tend to yellow and curl inward and, in many instances, growth of affected seedlings is arrested. However, some of the affected plants grow more rapidly than do healthy ones and are conspicuous in the field because of their height and etiolated appearance. The dry weight of the elongated seedlings was found to be significantly greater than that of the healthy plants. This overgrowth effect—occurring naturally—has been reported only in rice, although artificial inoculation has resulted in increased growth in maize, barley, sugar cane, sorghum, millet, wheat, and oats.

Kurosawa (1926) showed that the overgrowth or bakanae effect could be reproduced in rice seedlings by treating them with cell-free culture filtrates on which the causal fungus had grown. These filtrates reproduced all of the characteristic manifestations of the bakanae disease such as lengthening of the internodes and leaves, chlorosis (except under conditions of nutrient excess), and reduced tillering. This pioneer work of Kurosawa was followed by the isolation and chemical characterization of several biologically active substances. Two powerful growth-promoting substances, which were given the trivial names of gibberellin A and B, were isolated in crystalline form by Yabuta and Sumiki (1938), and Yabuta and Hayasi (1939a, b, 1940). It was found later that gibberellin A is converted to B by warming in dilute acid at 50°–70° C. Boiling under acid conditions converted both substances into a biologically inactive compound named gibberic acid. A third biologically active substance, gibberellin C, was isolated by Yabuta *et al.* (1941a) from acid-treated gibberellin A. The nature of the gibberellin skeleton was established by Yabuta *et al.* (1941b). It was demonstrated in these studies that gibberene obtained by selenium dehydrogenation of gibberellin A, B, or gibberic acid is a fluorene derivative.

Another quite distinct but chemically related, biologically active compound was isolated by American workers headed by F. H. Stodola and by British investigators under P. W. Brian. This substance was named gibberellin X by the Americans and gibberellic acid by the British group. The latter name is now commonly used for this compound. Although gibberellic acid, whose empirical formula is  $C_{19}H_{22}O_6$ , resembles gibberellin A in biological activity and degradation products, it differs from this compound in infrared spectra, optical rotation, in certain derivatives, as well as in the empirical formula. The basic skeleton of gibberellic acid, like gibberellin A, was found to be gibberene, which Mulholland and Ward (1954) identified as 1,7-dimethylfluorene. This led Cross *et al.* (1956) to suggest the tentative structure for gibberellic acid shown in Fig. 1. It is reasonably certain that the formula shown in Fig. 1 is essentially correct, although the exact points of attachment of the lactone grouping to the cyclohexenol ring of gibberellic acid are not known as yet.

Recently, Takahashi *et al.* (1955) reexamined gibberellin A and found this compound to be essentially homogeneous by countercurrent distribution, paper and partition chromatography. Esterification followed by chromatography on alumina led to the isolation of three methyl esters which were named gibberellin A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>. Stodola *et al.* (1957) believe that their gibberellin A is identical with A<sub>1</sub>. Gibberellic acid was shown to be identical with A<sub>3</sub>. Gibberellin A<sub>2</sub> has been isolated only in Japan

but Stodola (1956) reports its corrected empirical formula as  $C_{19}H_{26}O_6$ . Thus, the existence of three distinct gibberellins appears to be established.

Evidence is now available suggesting that the gibberellins elaborated by the fungus *Gibberella fujikuroi* correspond closely (in their biological activity) to growth-regulating compounds found to occur naturally in higher plants. West and Phinney (1957) have isolated—but have not yet identified chemically—an ether-extractable substance from wild cucumber seeds which, like the gibberellins of fungus origin, promotes the active growth of dwarf mutants of maize. Since these genetic dwarfs appear to be due to single-gene defects, they may be explained as resulting from blocks in the biosynthetic pathway leading to the formation of a natural gibberellin-like substance. The gibberellins appear to substitute in the plant for the missing product of that reaction. Very recently Mac-Millan and Suter (1958) have obtained high yields of gibberellin  $A_1$  from the seeds of runner bean plants. The isolation and characterization of gibberellin  $A_1$  from a higher plant indicate further that this compound, in all probability, participates directly in the growth-regulating system of higher plants and, therefore, represents a new type of endogenous growth regulator.

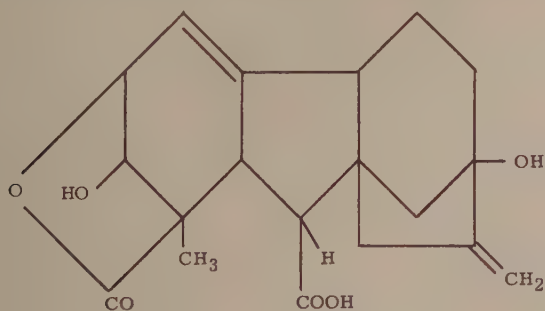


FIG. 1. Gibberellic acid.

The gibberellins of fungal origin have been applied to a large number of different plant species and have produced a variety of responses. The most consistent and striking response is a marked elongation of plant stems. In one survey of 42 different plant species belonging to many different families, only 3, white pine, gladiolus, and onion, failed to respond with stem elongation. In many instances, not only do the stems grow longer but also they are proportionately thicker. Gibberellin-treated oak and maple trees are not only taller but have a diameter more than twice as great as that of untreated control specimens. Brian and Hemming (1955) have reported remarkable stimulation of dwarf varieties of



peas, broad beans, and French bean by gibberellic acid. The treated dwarfs and untreated normal plants grew equally well.

In the bakanae disease of rice as well as in certain related diseases described earlier in this discussion, infected plants that reached maturity flowered earlier than did normal plants of the same variety. With the availability of pure gibberellic acid for experimental purposes, Lang (1956) found that a biennial variety of *Hyoscyamus* treated with that substance bolted and flowered in the first year. In a later study, Lang (1957) found that in annual varieties of *Hyoscyamus*, *Silene*, and *Samolus* the long-day requirement for flowering could also be replaced by gibberellic acid. Wittwer *et al.* (1957) found that gibberellic acid-treated lettuce bolted and flowered under photoperiodically adverse conditions. These same workers reported the gibberellins to be 500 times more effective than indoleacetic acid in inducing parthenocarpy in tomatoes.

Reduced fruit yields are characteristic of the bakanae disease. Hayashi *et al.* (1953) found that the gibberellins reduced rice grain production by 32% although the yield of straw was increased by 14%.

Stowe and Yamaki (1957) suggest that the one property common to the gibberellins is their capacity to remove certain limitations on cell enlargement, while incidentally releasing other responses. Despite this similarity to the auxins, the gibberellins are not auxins but appear to depend for their activity on the presence of auxins. The biological activity of these substances has been attributed (Brian, 1958) to the neutralization of a growth-inhibitory system which normally limits growth.

Certain other plant diseases give indication that infection interferes with the synthesis or utilization of gibberellin-like substances. Carrot plants infected with the tomato big-bud virus of California, as well as with a related virus found to occur naturally in carrots in the State of Washington, were shown by Kunkel (1951) to bolt and flower prematurely. These findings suggest that virus infection may increase the synthesis of gibberellin-like substances by the plant. On the other hand, in the rosette diseases, as exemplified by peach and peanut rosette, infection may interfere with the normal synthesis or utilization of these biologically active substances by the plant. The stunting effects, but not other symptoms associated with aster yellows, corn stunt, and wound tumor diseases, have in fact been reversed with gibberellic acid (Maramorosch, 1957).

## B. GROWTH CHANGES IN LEAVES AND PETIOLES

### 1. Growth Movements

Nastic responses refer to changes in position of a bilaterally symmetrical organ as a result of differential growth. Epinasty of leaf petioles

has been found by Wellman (1941) to be a characteristic early symptom of the *Fusarium* wilt disease of tomato plants. Hunger (1901) and Smith (1920) found it to be associated with Granville wilt, while Grieve (1941) recorded this condition as a primary symptom in roses affected with the rose wilt virus. Pronounced epinasty has also been produced experimentally by Locke *et al.* (1938) following inoculation of tomato plants with a highly virulent strain of the crown gall bacterium. Hypo-nasty, on the other hand, has been observed in plants infected with *Erwinia phytophthora*.

Grieve investigated the question of epinasty of the leaf petiole as it occurs in the Granville wilt disease (1936, 1939, 1940). In this instance, the growth response was found to be an irreversible one and the invasion of one lateral trace by the bacteria was sufficient to induce it. Grieve reported that a growth substance which appeared to be indoleacetic acid was elaborated by the bacteria in culture and that this substance induced a characteristic epinastic response when applied to tomato plants. However, virulent and avirulent cultures of the bacterium produced approximately equal amounts of the growth-promoting substance. Furthermore, no significant difference between the growth-substance content of comparable control and infected stem segments could be detected. Studies of the growth-substance distribution in upper and lower halves of reflexing petioles, on the other hand, showed a significantly greater concentration in the upper halves. In normal petioles, the concentration of growth substance was found to be greatest in the lower halves of the petioles. Grieve (1939) reached no definite conclusion as to how the redistribution of growth substance in the basal part of the petiole is accomplished. He pointed out (Grieve, 1943), however, that the balance of the normal growth-controlling mechanism at the base of the petiole is very delicate, as evidenced by the fact that ethylene, in one part in ten million parts of ambient air, as well as very minute amounts of growth substance from bacterial cultures will disturb it. Grieve considers that even a small stimulus from the invading organisms can initiate a chain of reactions that leads to a redistribution of growth substance with a resulting nastic response.

More recently, Dimond and Waggoner (1953) made a detailed investigation of the cause of epinastic symptoms in *Fusarium* wilt of tomato plants. From these studies it was concluded that ethylene is responsible for the characteristic growth response. This conclusion was based on the finding that ethylene is produced in culture by the causal agent of this disease, *Fusarium oxysporum* f. *lycopersici*. Ethylene production by infected tomato plants was demonstrated, moreover, by confining such plants with healthy indicator plants. Under these conditions, epinastic responses developed to a greater degree than when the indicator plants

were confined with healthy tomato plants. In these studies, ethyl alcohol, which is capable of causing epinasty in tomato and which is produced by the causal fungus in culture as well as in the infected host, was also considered a possible cause of the growth response. This compound was ruled out, however, because the amounts produced by the fungus in the hosts were insufficient to account for the observed epinastic responses.

Another characteristic response—involving growth movements—which is associated with certain disease conditions is concerned with the upright growth habit of leaves. This is one of the most characteristic symptoms of the aster yellows disease in many different plant species. Leaves on a normal aster plant take approximately a horizontal position in relation to the main axis of the stem. In virus-infected plants, on the other hand, the petioles elongate and the leaves assume an extremely upright habit of growth which approximately parallels the main axis of the plant. It is this upright habit of growth that suggested the name "rabbit ears" for lettuce plants infected with the aster yellows virus.

Kunkel (1954) has shown that twigs of peach trees infected with peach yellows virus characteristically assume an upright growth habit. In this instance, the plants can be cured of the disease by thermal treatment. After cure, the new growth of such twigs again takes a normal position with respect to the main stem. This change necessitates a change in the direction of growth of the terminal bud.

## 2. Curling and Distortion

The curling and distortion of leaves are characteristic of many diseases. Needless to say, such well known maladies as curly top of sugar beet, peach leaf curl, and leaf roll of potatoes have been assigned their trivial names because curling of leaves is the most conspicuous symptom of the disease. The most extreme form of leaf rolling is found in the so-called scroll galls produced by certain insects. Under the influence of specific insects, the leaves curl lengthwise to form tightly rolled scrolls in which the insects live.

Peach leaf curl caused by *Taphrina deformans* is perhaps the most studied of this type of disease. The causal fungus commonly infects very young leaves which soon become either quite red or paler in color than are the normal leaves. Such infected leaves soon become curled and puckered, increase greatly in thickness, and have a firm consistency. The host cells which are in contact with the invading fungus are stimulated to abnormal activity. This may involve isolated regions of a leaf or in extreme instances most of the leaf. Such cells increase in size and number and produce marked changes in the form and structure of the leaf. Loss of chlorophyll is almost complete in the stimulated cells. The

increase in size of the cells on either side of the midrib results in a puckering of the leaf. At the same time, the leaves tend to curl and become concave on the lower side. The cells of the palisade parenchyma respond much more actively to the stimulus of the fungus than do the subjacent cells of the spongy parenchyma, thus producing the curling. Link *et al.* (1937) have reported that a substance having the properties of an auxin and which was presumably indoleacetic acid was extractable from *Taphrina deformans* culture filtrates. The role, if any, that this substance plays in the development of the disease picture as described above is as yet not clear. It is nevertheless true that auxins are capable of stimulating cell enlargement of the type described above in parenchymatous cells of many different plant species.

### 3. *Frenching and Shoestringing*

Frenching, a well-known deformity of tobacco, occurs in most tobacco-growing regions of the world. This condition is, in an advanced stage, characterized by a cessation of terminal bud and stem growth, and by a reticular type of chlorosis in the slowly expanding new leaves. This chlorosis may disappear as the young leaves develop and become strap-shaped (sword and string) as a result of the failure of the leaf lamina to expand. As apical dominance in such diseased plants is lost, the axillary buds develop and an unusually large number of leaves—which may be as high as 300—appears on a plant. These leaves assume an upright growth habit and are commonly sword- or string-shaped. Such plants have the appearance of a rosette or, in extreme instances, of a witches' broom. Root growth is also somewhat inhibited, although the effect on the leaves appears to be the most characteristic feature of the disease.

The extent to which frenching modifies the morphology of a leaf can be seen from some figures that are given by Wolf (1935). Normal tobacco leaves average 55 cm. in length and 30 cm. in width. The corresponding measurements for sword leaves and string leaves are 40 cm.  $\times$  10 cm., and 27 cm.  $\times$  1.8 cm., respectively. Histological studies show, moreover, that the diseased leaves are two to three times as thick as normal leaves. This increase in thickness is due to an enlargement of cells of all the leaf tissue except cells of the vascular system. Schweizer (1933) has indicated that the xylem is markedly reduced in development in frenched leaves. In mature string leaves, the parenchyma remains quite juvenile with little or no evidence of dorsiventrality.

Although the cause of frenching is not clearly established, it is now believed to be a toxicity disease rather than the result of a nutritional deficiency. No parasitic organism of any kind has yet been implicated



in this condition. McMurtrey (1932) described briefly the similarity of frenching symptoms to those of thallium toxicity in tobacco. Spencer (1935) found that one part of soil obtained from a field in which frenching occurred mixed with 2,000 parts of sand produced typical frenching in the test plants. Thus, a toxic factor, effective in very low concentrations, was present in frenching soils. Spencer (1935) tested 33 different elements on tobacco and found, as had McMurtrey, that only thallium at a concentration of 5 p.p.m. or less produced chlorosis, strap-shaped leaves and other symptoms characteristic of frenching. Later, Spencer and Lavin (1939) indicated that frenching and thallium toxicity are probably two distinct physiological conditions.

Steinberg (1947, 1950) has suggested that frenching of tobacco is caused by the action of diffusates from the presumably nonpathogenic soil bacterium *Bacillus cereus*. The effectiveness of *B. cereus* diffusates in eliciting typical frenching symptoms in tobacco varied with the kind and quantity of peptone used and the concentration of inorganic nitrogen in the test medium.

*Bacillus cereus* is a widely distributed soil microorganism. The progressive development and type of symptom produced by it in tobacco (in aseptic culture) largely parallel those that appear in plants subject to this abnormality under field conditions. Moreover, rhizosphere and rhizoplane counts of this organism increased by 65% and 200%, respectively, when frenching occurred in the field. The nature of the toxic substance is not yet known.

Steinberg (1952) has shown that slight excesses of certain amino acids caused production of symptoms resembling frenching in tobacco seedlings. Frenching symptoms were, however, limited to the natural isomers of alloisoleucine and isoleucine in tobacco and to these compounds and leucine in *Nicotiana rustica*. Alloisoleucine was most effective in both species and as little as 2 to 8 p.p.m. resulted in chlorosis and strapping of leaves in tobacco. Leucine, which was ineffective when applied to tobacco, was more effective than isoleucine in *N. rustica*. Analytical studies indicated, moreover, that frenching was found to be accompanied by a marked increase in isoleucine and other free amino acids in the leaf lamina of field-grown plants (Steinberg *et al.*, 1950). The absence of free amino acids in frenching soil and the marked increase of free isoleucine in frenched leaves indicate that *Bacillus cereus* toxin and isoleucine are not identical. The conclusion was therefore drawn that the accumulation of excessive quantities of free amino acids in the strapped leaves was a probable chemical factor involved in the production of the morphological symptoms in the plant. The stages suggested by Steinberg (1952) leading to abnormal growth in frenching of

field tobacco were, therefore, as follows: bacterial soil toxin → receptor → excessive accumulation of isoleucine and other free amino acids in the leaves → frenching.

A condition resembling frenching may also be caused by certain strains of the tobacco mosaic and cucumber mosaic viruses or a mixture of these viruses, as well as by a gene mutation. Kunkel (1954) has reported an extreme instance of shoestringing caused in tomato plants by a mixture of tobacco mosaic virus and the virus of carrot yellows of Texas. Leaf-blade development in this instance was completely suppressed. Just how these viruses suppress or prevent leaf-blade growth is not understood.

The so-called "wiry" tomato plants described by Lesley and Lesley (1928) are not unlike those which result from the virus diseases of the shoestring type reported above. Wiry plants appear, however, to result from genic mutation. This condition is recessive to the normal and the plants are completely sterile. The leaves of these plants are variable in shape but have a strong tendency toward reduction of the leaf lamina and in extreme instances the leaves consist merely of a tapering midrib.

### C. Growth Changes in Stems and Branches

#### 1. Organs Arising in Unusual Places

a. *Adventitious Roots.* The development of adventitious roots on stems of plants has been reported to be a characteristic host response associated with certain bacterial, fungal, and viral diseases.

A number of investigators (Bryan, 1915; Grieve, 1936, 1940; Hunger, 1901; Smith, 1914, 1920) have described this phenomenon following infection of tomato and certain other hosts with *Pseudomonas solanacearum*. Smith (1914, 1920) reported it to be associated with the Grand Rapids disease of tomato which is caused by *Corynebacterium michiganense*. Adventitious root formation was found to occur on tomato (Locke *et al.*, 1938) and *Kalanchoe* (Price and Gainor, 1954) following inoculation of those host species with the crown gall bacterium. It has also been observed by Wellman (1941) and Dimond and Waggoner (1953) in tomato plants diseased with *Fusarium* wilt. Certain viruses, such as the cranberry false blossom virus in tomato as well as that implicated etiologically in the sereh disease of sugar cane, characteristically stimulate root formation in the stems of their respective hosts.

Grieve (1936, 1940) studied the development of adventitious root formation in tomato plants infected with *Pseudomonas solanacearum*. This investigator found that the new roots arise commonly over a span of several internodes along the path of the primary bundle except in

those instances in which the disease progresses with great rapidity, in which case these adventitious structures do not develop. Histological examination of transverse and longitudinal sections of infected plant stems demonstrated that adventitious roots usually develop in regions where large primary bundles are affected. The initiation of root primordia often precedes the advancing bacteria, indicating action at a distance. Grieve applied indoleacetic acid to the stems of tomato plants and found that the roots initiated were similar to those resulting from infection. This finding suggested that the bacteria are inducing root formation either directly as the result of the production of a growth-promoting substance, or indirectly through their interference with the metabolism of the plant. In attempting to distinguish between these two possibilities, Grieve found that *Pseudomonas solanacearum* produces indoleacetic acid from tryptophan in culture. However, virulent and avirulent strains produce approximately equal amounts of this substance. The possibility that the bacteria produce an auxin in the xylem by acting on naturally occurring or artificially introduced tryptophan was examined but no evidence for such production was obtained. No difference in auxin concentration could be detected, moreover, between healthy and diseased plants by bioassay methods. Furthermore, adventitious roots could be produced by cutting or blocking the bundles. These findings led Grieve to question whether the bacteria induce the formation of adventitious roots directly through the elaboration of indoleacetic acid. He suggested (Grieve, 1943) that the production of these structures more likely results from disturbances of normal auxin transport in the plant as a result of mechanical blocking of the vessels by the bacteria.

Price and Gainor (1954) reported a striking correlation between adventitious root development and the number of leaves present on a *Kalanchoe* plant inoculated with crown gall. These workers suggested that a substance responsible for stimulation of adventitious roots is organized in leaves and is transported downward to the site of the tumor. That the biologically active substance is not indoleacetic acid was indicated by the finding that 2% of this substance in lanolin—applied to stumps of petioles above the points of inoculation—did not stimulate the development of adventitious roots in *Kalanchoe daigremontiana*.

De Ropp (1947a) has shown, moreover, that a powerful root-stimulating substance is elaborated by sterile crown gall tumor cells. In crown gall it appears likely, therefore, that the stimulus for adventitious root formation originates in the cells of the host rather than directly from the causal bacteria.

The course of *Fusarium* wilt of tomato is not unlike that of the bacterial wilt of this host as described above. Wellman (1941) reported

adventitious roots associated with this fungus disease. Since epinasty of the leaf petioles often appears on plants showing adventitious root formation, Dimond and Waggoner (1953) have suggested that in the case of *Fusarium* wilt, adventitious root formation, like epinasty, may result from the production of ethylene either by the causal fungus or by the cells of the host as a result of the interaction of the host and the pathogen.

b. *Adventitious Shoots*. The witches'-broom virus in potato causes the infected plant to produce numerous buds at the nodes in the above-ground stems of potato plants. Long slender stolons that resemble aerial roots but which are covered with trichomes develop from these adventitious buds. Aster yellows virus and carrot yellows virus from Texas, on the other hand, stimulate the production of small aerial tubers in the axils of the leaves of potato plants.

An extreme example of adventitious shoot formation was reported about 100 years ago by von Martius. This worker described an unusual plant that possessed a mania for forming innumerable leaves and shoots. This species has been appropriately named *Begonia phyllomaniaca*. Plantlets develop spontaneously in incredible numbers from the superficial cell layers of the leaf lamina, petioles, and stems. Erwin Smith (1920) studied phyllomania and believed it to be conditional on shock such as is encountered during the repotting of plants. Smith indicated, moreover, that the cells of leaves and internodes are susceptible to such shock only during a relatively brief period of meristematic growth. The adventitious shoots do not arise from preformed buds but develop from totipotent cells at the base of the trichomes and especially from botryose glands which are found in great abundance in young stems and leaves of this species. These embryo plants develop a vascular system of their own but the vast majority never succeed in connecting this with the vascular system of the plant. They must, therefore, be considered not as branches but rather as independent organisms.

## 2. *Witches'-Brooms*

Witches'-brooms, or "hexenbesens," are closely grouped, much branched structures commonly found to occur on a number of different species of trees and shrubs. The stimulus necessary for their formation is supplied in different plant species by pathogens of the most diverse types. Fungi of the genus *Taphrina* and various rust fungi are effective in inducing witches'-broom, while distinct virus species have been shown to cause the formation of such an abnormality in alfalfa, potato, peach, and the black locust. Bos (1957) has recently presented a detailed account of the virus-induced witches'-broom. An eriophyid mite belong-



ing to the genus *Aceria*, followed by a powdery mildew fungus, is said to be involved in the development of branch knot so commonly found on the hackberry.

Typical witches'-brooms caused by the fungi appear to live a more or less independent life and act as parasites on the plants from which they are derived. In accordance with their independent existence, witches'-brooms tend to break away from the correlations of the parent plant. Instead of branching out horizontally, the brooms stand as more or less erect clusters of branches. A normal dorsiventral symmetry is thus changed to a radial symmetry. Witches'-brooms as a rule do not produce flowers, indicating further a breakaway from the correlations of the parent plant. Heinricher (1915) has shown, moreover, that a twig of a witches'-broom caused by *Taphrina cerasi* grafted to a healthy sweet cherry tree develops again into a typical witches'-broom. The independence of these structures is further shown in an impaired periodicity. The vegetative buds found on the brooms of the sweet cherry, for example, open several weeks earlier than do those present on healthy branches (Schellenberg, 1915). Gäumann (1946) has pointed out that this premature unfolding of the buds is probably associated with the fact that the shoots and buds comprising the broom never achieve a true winter dormancy since the pathogen never becomes entirely quiescent. This incomplete winter dormancy may result in a winter killing of the first year twigs present on a broom.

The witches'-broom of the silver fir caused by *Melampsorella caryophyllacearum* is typical of this peculiar type of growth. The primary infection is said to occur in the young bark of branches surrounding buds. In the spring when the buds develop, the fungus mycelium grows into the epidermis of the developing shoot and penetrates the cortex so that by fall a slight swelling of the shoot axis is found. During the following year, an overgrowth of considerable size may be formed and buds embedded in this growth develop to produce the characteristic deformation. The twigs of this particular type of broom are found to develop in whorls. They are short, thick, soft, and pliable. This results from the fact that the cortical parenchyma is spongy and the wood is not well developed. The buds on the broom open earlier in the spring than do those present on a healthy twig, while the leaves found on the diseased specimens remain short, are yellowish in color, and fall off when a year old. The leaves present on a normal twig are, on the other hand, long, straight, dark green on the upper side, and commonly remain in position 5 or more years. The longevity of the diseased twigs themselves is limited and they die within a few years, giving rise to the dry, bristling brooms characteristically found on the silver fir.

The witches'-broom-like effects that occur in certain plant species following virus infection appear to result from the excessive stimulation and development of secondary shoots. This condition is not accompanied by swellings or overgrowths characteristic of many of the fungus-induced brooms.

Brian (1957) has suggested that the diseases characterized by excessive branching may well be due to auxin deficiency rather than to auxin excess. Lacey (1948) has, in fact, shown that cultures of *Corynebacterium fascians*, the causal agent in leafy gall development on certain plants, are capable of rapidly destroying indoleacetic acid and other auxins present in plant tissues.

#### D. Growth Changes in Floral Organs

##### 1. Alterations of Floral Parts

Under the stimulus of pathogens, the sepals, petals, stamens, or pistils of a flower may be transformed into structures that are very different in appearance from those found normally. Kerner von Marilaun (1891) has reported that double flowers are produced in *Valerianella carinata*, the common corn salad, as a result of infestation by a mite. This doubling results from a retrograde alteration of the stamens into a whorl of petals. These petals, under the stimulus of the mite, enlarge to more than fifty times their original size and finally appear as fleshy lobes which fuse with one another into a disc. The greatly enlarged lobes bend backward and are concave on the lower side. It is in the cavities thus formed that the gall mites live.

In the capitula of certain of the milfoils, *Achillea millefolium* and *A. nana*, the peripheral ray florets and the central tubular ones become leaf-like in appearance and assume remarkable forms as a result of mite infestation. A capitulum is often subdivided into several stalked sub-capitula, while the flowers are altered into green funnel-shaped structures with jagged mouths and into small flat-lobed green foliage leaves, while short green scale-like leaflets, which represent modified stamens, develop from the midribs of these leaves. Sometimes, however, the changes in growth pattern following mite injury are not so extreme as the two instances cited above might suggest. In *Veronica saxatilis*, for example, the development of numerous hairs on the rachis of the raceme, the pedicels, and the bracts appears to be the only characteristic host response. Hairs are not present in the corresponding normal structures of this plant species.

Doubling of the flowers of the alpine rose, *Rhododendron ferugineum*, has been reported by Kerner von Marilaun (1891) to result

from gnat infestation. In this instance the stamens and carpels are transformed into red petals. Since flowers of this species normally have 10 stamens and 5 carpels, there should be only 15 red petals in the center of each but there are often two or three times that many present. It thus appears that not only metamorphosis but multiplication takes place.

According to Tschirch (1890), the aphid *Astegopteryx stryacophila* induces remarkable changes in young flowers of *Styrax benzoin*. The calyx, corolla, and androecium are transformed into large abnormal leaves that form bag-like pockets. The pistils, however, appear to be unaffected in this instance.

Metamorphoses of floral parts of higher plants are also brought about by certain species of fungi. The transformation of stamens into petals commonly follows infection of *Viola sylvestris* by *Puccinia violae*. Similarly, *Peronospora violacea* has been reported to encourage stamen primordia in the flowers of *Knautia arvensis* to develop into petal-like structures. An extreme instance of phyllody is found when flowers of the Japanese plum are infected with a rust of the species *Caeoma makinoi*. In this instance all of the floral parts are transformed into foliage leaves. In the alder the bracts of the pistillate flowers are changed by *Taphrina alni-incanae* (*Amentorum*) into greatly elongated purple-red spatulate lobes which are twisted and bent.

Von Tubeuf (1895) has reported that when *Albugo candida* infects the inflorescences of the radish, the ovary, calyx, and corolla enlarge and the androecium assumes a leaf-like appearance. Stamens may become green and leaf-like in the downy mildew disease of *Pennisetum glaucum* and other grasses. Thus, this disease, which is caused by *Sclerospora graminicola*, is popularly known as green ear. In the case of head smut of maize, caused by *Sorosporium reilianum*, the whole staminate head or the ear may become a leafy structure.

In the United States, there are at least three different viruses, the aster yellows virus, the cranberry false blossom virus, and tomato big bud virus, that produce gigantism in the floral organs and more particularly in the sepals and calyxes of tomato and certain other solanaceous plants. The tomato big bud virus appears to affect the sepals rather specifically. These structures enlarge greatly under the influence of this virus and fuse to form huge bladder-like growths that conceal the inner parts of the flower. The stamens and pistils do not appear to be greatly affected. The aster yellows virus and the cranberry false blossom virus cause symptoms in the flower trusses of tomato that are almost identical with those caused by the big bud virus. Although these three viruses cause the sepals of certain of the solanaceous plants to enlarge greatly, they

do not have this effect on sepals of species of certain other families. They do not, for example, cause gigantism in the sepals of the flower trusses of the periwinkle, *Vinca rosea*, but they bring about a vireescence in the petals, stamens, and styles. There is, in this instance, a retrograde development of floral parts into foliage leaves. It is, therefore, clear that the type of symptom produced depends as much on the species of plant in which the virus multiplies as on the nature of the virus.

The aster yellows, cranberry false blossom, and carrot yellows virus of Texas stimulate the formation of adventitious buds in the stigmas of flowers of a number of different hosts. Such buds may give rise to either flowers or to leafy stems which in turn may bear flowers.

Bos (1957) has recently attempted to interpret the development of the flower and its component parts on the basis of the phenomenon of antholysis accompanying virus infections in *Crotalaria* and certain other hosts. This investigator has suggested, on the basis of the homology of the reproductive and vegetative parts of a plant and their morphogenetic development, that the sexual and vegetative activities in the plant are mutually antagonistic. A growing point can develop only into an inflorescence or into a vegetative shoot. During flower initiation the vegetative characters are suppressed and sexual characters prevail. The sexual characters remain suppressed, on the other hand, during vegetative growth. From the manner of appearance of antholysis in virus-infected *Crotalaria* plants, Bos has concluded that flower induction is stopped suddenly and that subsequent development of the floral parts proceeds exclusively in a vegetative manner. The ultimate result obtained depends upon the stage of development of the primordia in the bud at the time of suppression of flower induction by the virus. Thus, flowers initiated in succession, produce a series of flowers showing increasing antholysis. These—in reverse order—clearly show a macroscopically recognizable picture of the morphogenesis of the flower and of its component parts. Therefore, according to Bos, antholysis supports the theory that the flower must be regarded as a modified leafy branch.

## 2. Overcoming of the Normally Arrested Development of Floral Parts

The anther smut, *Ustilago violacea*, produces its spores only on the anthers of certain hosts. When this fungus infects the female flowers of *Melandrium album* or *M. dioicum*, the stamens, which are normally arrested in their development and are present only in a rudimentary form in the pistillate flowers, grow to full size but when mature are filled with smut spores instead of pollen.

A similar process is said to occur when pistillate flowers of *Knautia*



*arvensis* and *K. sylvatica* are infected with *Ustilago scabiosae*. The nature of the morphogenetic stimulus which overcomes the arrested development of male sex organs in female flowers is still unknown.

### E. Modification in Fruiting Structures

Modification of the fruiting bodies of higher plants occurs quite commonly as a result of infection. Such descriptive names as little peach, bladder plum, phony peach, etc., have been applied to conditions of this type. Hypertrophied fruits are commonly produced in certain species of the genus *Prunus* as a result of infection by *Taphrina pruni*. In this instance, the tissue of the diseased ovary is stimulated to growth, but not in the same way as in the normal fruit. The resulting body is flattened on two sides, is brittle, yellow in color, and much longer than the normal fruit. The seed within is abortive and a hollow space is left in its place. These hypertrophied growths, which are commonly called "bladder plums," fall from the trees at the end of May and are said to be eaten in certain areas. A somewhat similar condition is found when *Taphrina aurea* infects the pistillate flowers of the poplar. This fungus, like the one described above, stimulates the growth of the ovaries with the resulting development of golden yellow capsules that are more than twice the normal size. The smut fungus *Ustilago zeae* also stimulates growth of the tissue of the pistillate flowers of maize. As a result, the grains are replaced by irregular cushion-like structures with a diameter of up to 7 cm. The resulting growths contain more auxin than do normal tissues. *Ustilago zeae*, moreover, produces an auxin in culture (Moulton, 1942) which was identified as indoleacetic acid by Wolf (1952). The fruit size may be greatly dwarfed as in the case of two virus diseases, little peach and phony peach, or the fruits may assume an asymmetrical shape as is found characteristically in the xyloporosis disease of oranges—which is also of viral origin.

The seed capsules of *Datura stramonium* normally bear numerous conspicuous spines. When plants of this species are infected with the severe etch virus, spine formation may be completely inhibited (Kunkel, 1944). The aster yellows virus, on the other hand, causes a bursting of the abnormal fruits produced by *Cajophora lateritia* plants infected with this agent. The seed-like structures present in these fruits are green in color and have been transformed, under the influence of the virus, into short stems which bear leaves.

The entire inflorescence may, in certain disease conditions, be altered to quite a different type. The rust fungus, *Aecidium esculentum*, is said to cause the normal inflorescence in *Acacia* to change from a head to a spike. Similarly, the stinking smut fungus, *Tilletia tritici*, may change the

growth pattern of the head of the club types of wheat to the elongated or "vulgare" type.

Individual floral parts or even entire organs may be replaced by new structures in certain diseases. *Claviceps purpurea* invades and destroys certain of the ovaries of rye and other grasses. In place of the seeds, elongated dark purple sclerotia are produced. These are composed of dense aggregates of fungus mycelium.

In sheep sorrel infected with *Ustilago oxalidis* the seeds are replaced by the spores of the pathogen. In this instance, the spores are forcibly expelled from the seed capsule as if true seeds were present.

### F. Fasciation

A condition known as fasciation can be classified between that group of growth abnormalities described above (involving harmonious changes in growth pattern) and the amorphous changes to be considered later in this discussion. Fasciation is a morphological term that has been used to describe a series of abnormal growth phenomena resulting from many different causes, any of which result at the morphological level in a flattening of the main axis of the plant. Although this ribbon-like expansion of the stem is often the most striking feature of this condition, all parts of the plant may be affected. Fasciation often results in alterations in the arrangement of foliar and floral structures. White (1948) points out that, when fasciation occurs, the early seedling growing stages are normal. As the plant develops, however, the growing point becomes broader, and the unregulated, distorted tissue growth results in significant increases in weight and volume of plant tissue. The apical growing region becomes linear and comb-like in some instances or develops numerous growing points, producing a witches'-broom effect. In still other instances, the growing points may be coiled and resemble a ram's horn or they may be highly distorted into a grotesque tangle of coils. Fasciations are widespread, both geographically and taxonomically. Examples have been recorded in 102 families of vascular plants. Fasciations found in certain plant species such as the common cockscomb, *Celosia cristata*, as well as the cristates in the cacti are highly prized by gardeners.

Fasciations have been classified on morphological grounds—based on such physical features as form, color, and anatomical structure. Linear, bifurcated, multi-radiate, and ring or annular types have been listed (de Vries, 1909–1910; White, 1948). This condition has also been classified from a causal standpoint. In this type of classification the conditions and agencies necessary to produce the character as well as the modifying factors that affect its development are considered. White (1948) has placed these essentially into five categories (1) fasciations which breed

true and which in crosses with the normal obey genetic laws so that their genic basis is known; (2) noninherited forms in which the fasciation is due to environmental causes and the character is not reproduced in the selfed seed; (3) fasciations that occur spontaneously and which have been propagated vegetatively but in which neither the initial cause nor the question as to whether the condition is transmitted through the seed is known; (4) fasciations which are induced artificially by known procedures; (5) fasciations which have been imperfectly investigated.

Fasciations resulting from modifications of the normal gene complement, and which breed true, have been known for centuries in such plants as *Celosia* as well as in the mummy or crown type of garden pea. Gregor Mendel (1866) found fasciation in the mummy pea to be recessive in the  $F_1$  when crossed with normal varieties. Such  $F_1$ 's showed a ratio of 3 normals to 1 fasciate in the  $F_2$ . A gene-fasciated race in *Nicotiana tabacum* when crossed with nonfasciated strains, on the other hand, gives 1:2:1  $F_2$  ratios. In other instances, very complicated results were obtained, showing the effects of modifying factors and giving rise to nonfasciated segregates which carry the fasciated gene. Fasciation is not correlated with changes in chromosome numbers but it may produce meiotic irregularities.

Aside from gene mutation, fasciation has been reported to result from environmental effects such as frost, pressure, alteration of food and water relationships, pruning, mutilation, as well as by more specific etiological agencies such as a bacterium, *Corynebacterium fascians*, and X-irradiation.

There seems little doubt but that nutritional changes due to correlative disturbances in growth-substance relationships play a role in fasciation. Bloch (1938) has reported that this condition can in certain instances be produced experimentally by treating plants with auxin-containing pastes. On the other hand, high doses of X-rays have been shown to inactivate auxins and yet disturbances caused by them also result in fasciation. The effects of X-irradiation on auxin metabolism in the growing plant are obviously complex. These nongenetic types of fasciation are not transmitted through the seed but may, in some instances, be propagated asexually. Both inherited and noninherited types of fasciations which are phenotypically indistinguishable have been described for at least 3 different plant species.

Orland E. White believes that the basic cause of fasciation is a disturbed metabolism involving excessive nutriment which mobilizes energy that must be used. This energy, once accumulated, goes, according to White (1948), into growth and becomes "wildly" expended in abnormal and unpredictable tissue production. Heslop-Harrison (1952) points out,

however, that these conclusions are descriptive rather than truly explanatory since excessive nutrition in itself cannot account for the abnormal element in fasciation. He believes, rather, that a proximate cause of these abnormalities is probably to be found in maldistribution of the auxins in the plant. Jones (1935) and Orland White (1948) have suggested that fasciation bears an analogy to animal cancer. In the opinion of the writer, however, this condition does not represent a true tumor at all but might better be placed in that category of teratological abnormalities known as monstrosities.

### III. AMORPHOUS CHANGES IN GROWTH PATTERN

Amorphous changes involving either the temporary or permanent loss of typical organization are found to occur very commonly in plants. These overgrowths range from somewhat exaggerated but self-limiting wound-healing responses at the one extreme, to rapidly growing non-self-limiting tumors which have no characteristic size or structure, at the other.

#### *A. Self-Limiting Overgrowths*

##### *1. Intumescences*

Intumescences, which are among the simplest and most innocuous of this type of growth, are found to occur most commonly on leaves but are also found on stems and fruits of plants. These blister-like pustules usually result from the abnormal elongation of groups of cells with or without increased cell division. In a typical leaf intumescence found in many plant species, the palisade parenchyma cells elongate considerably in localized areas to give rise to the pustules. Intumescences have been found to be produced experimentally in a number of ways. No pathogens, however, appear to be concerned in their development. Sorauer (1886) believed them to result from an excess of moisture in the air and soil. Harvey (1918) produced intumescences artificially on the undersurface of cabbage leaves by subjecting such leaves to slight freezing. Smith (1920), on the other hand, induced blister-like pustules in cauliflower leaves with the use of such irritating chemicals as formic and acetic acids. Wolf (1918) has shown that intumescences may be produced in cabbage leaves by means of a sandblast and this worker has indicated that such overgrowths found naturally in the field result from sand driven against the leaves by wind. More recently, La Rue (1933a, b, 1935) was able to reproduce perfectly the intumescences which arise under conditions of high humidity on the leaves of poplar with the use of low concentrations of indoleacetic acid. Much larger self-limiting over-



growths were produced artificially by Brown and Gardner (1936) on bean plants with the use of higher concentrations of that substance. It is not unlikely that certain of the burls as well as the overgrowths that sometimes arise at graft unions result from a hormonal imbalance of this type.

## 2. Galls

Localized overgrowths in which the host cells are stimulated to excessive growth by pathogens are known as galls. In these instances, the continued growth of the host cells is dependent upon continued stimulation by the pathogen. There are unusually large numbers of distinct overgrowths of this type to be found in plants of which only a few representative examples have been selected for discussion.

a. *Insect Galls*. Among the most interesting types of self-limiting growth abnormalities found in plants are those that result from the activity of certain of the gall-forming insects. The whole subject of insect galls as reviewed in detail by Küster (1911), Ross and Hedicke (1927), and more recently by Felt (1940) suggests that many of these highly specialized overgrowths represent beautiful examples of dependent differentiation.

Insect galls may result either from a mechanical or chemical stimulus. Stem swellings on roses caused by the closely placed spiral galleries of the rose stem girdler, *Agrilus viridis*, are believed to be an example of the former type. Chemical stimulation, however, is probably far more important than mechanical irritation in the production of many insect galls, although the chemical stimulus may in some instances be supplemented by directive feeding of the insect. An examination of the literature leaves the unmistakable impression that highly specific morphogenetic stimuli, of chemical nature, and elaborated by insects, are capable of initiating, stimulating, and directing most precisely the development and differentiation of plant cells. There is no question about the fact that the morphological form that a gall assumes depends upon the nature of the pathogenic insect. This is evidenced by the fact that the same host species or even the same organ of the same plant attacked by different but closely related insect species produces morphologically very different galls. Figure 2 illustrates this point and shows drawings of 4 galls of quite different morphology induced on leaves of the California white oak by 4 closely related species of cynipids. The morphology of the gall is so specific that it is considered by some to be a more reliable criterion for distinguishing between closely related species of insects than are the morphological characters of the adult insects. The morphology of the gall does not, moreover, appear to be a function of the part

of the plant from which it arises. Currant galls of the oak, which are found on both flower stalks and leaves, have similar morphological structure at both points of origin. The same insect species may, moreover, produce the same gall type on different species of plants. The sawfly, *Micronematus gallicola*, produces bright red galls of similar morphology on four different species of willow. Findings such as those reported above suggest in the strongest possible manner that a large number of highly specific chemical substances are elaborated by insects and that these substances have specific morphogenetic effects on the cells

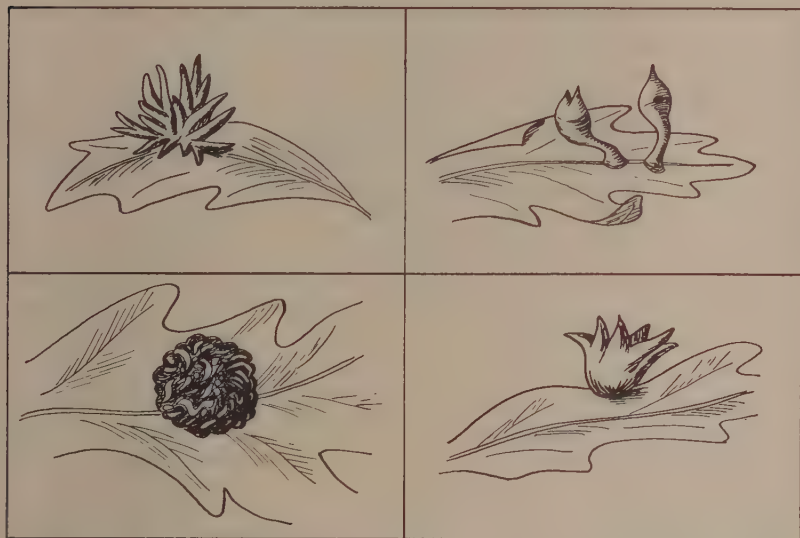


FIG. 2. Four morphologically distinct galls produced on leaves of the California white oak by four closely related species of insects. (Drawings by R. J. Mandlebaun.)

and tissues of a plant. However, only moderate success has thus far been achieved in establishing experimentally the truth of this view. That a morphogenetic stimulus is elaborated by certain insects is suggested by the fact that the Norway spruce aphid, *Adelges abietis*, attaches itself firmly by its beak to the scale of a fir bud and can directly influence only a few cells of the young shoot. Nevertheless, thousands of cells on this shoot are affected and soon begin to assume an altered form and are stimulated to growth, indicating that a diffusible stimulus is active. Plumb (1953) has, in fact, induced the formation of this gall by injecting a glycerol extract of the salivary glands of the insect into the bases of spruce buds. Beijerinck, as early as 1888, killed the eggs of a sawfly with

a hot needle immediately after they had been deposited in the host and found that the galls developed normally. Beijerinck called the biologically active materials "growth enzymes," and in 1897 applied this concept to the development of form in general. Parr (1940) showed that the gall-forming coccid, *Asterolecanium variolosum*, stimulates the host tissue to both hyperplastic and hypertrophic activity. He demonstrated further that the effect of the salivary secretion of this homopteron continues to stimulate plant cells even after the insect itself is gone. It was possible to reproduce galls similar to those produced by the insect by injecting glycerol extracts of the salivary glands. When the salivary extracts were heated to 60° C. they did not produce galls, indicating that a heat-labile, enzyme-like substance was involved in gall formation.

Rössig (1904) investigated various organs of gall wasps and believed that he could trace the site of formation of the gall-forming substances to the Malpighian vessels. Similar findings were reported by Triggerson (1914) for the cynipid which produces the oak-hedgehog gall. Lewis and Walton (1947) reported some very interesting histological and cytological findings dealing with a biologically active substance believed to be involved in the growth and differentiation of cell development of the cone gall of witchhazel. This gall is produced by the aphid species *Hormaphis hamamelis* Fitch. In this instance the stem mother inserts her stylets into and between the cells of an immature leaf and injects very minute droplets of a substance secreted by glands opening into the stylar canal. This stinging process is not to be confused with feeding and the sting substance is quite different in its nature from substances found in the salivary secretions. When about 150 stings have been made in a small circular area by the stem mother, the cells in this region at first become etiolated. Almost immediately thereafter, cells receiving injected sting material dedifferentiate, undergo rapid mitotic divisions, and then redifferentiate into gall cells rather than typical leaf cells.

The injected sting material consists of a ground substance in which minute crystalloids are embedded. These crystalloids show a reddish purple cast in such stains as gentian violet, Congo red, and acid fuchsin. They are capable of passing readily from cell to cell as well as of entering cells when they are deposited by the insect in the intracellular spaces. Whether the injection is inter- or intracellular, the end result is the entry of the crystalloids into the nucleolus. There they may fuse to form a large crystalloid which again breaks up into smaller ones as mitosis begins. During nuclear division, the crystalloids are distributed to the daughter nuclei where they are again found in the nucleolus. Since these bodies are incapable of self-propagation and since they are apparently used up during growth of the cells, fresh sting material must be injected

repeatedly by the stem mother during the entire growth of the gall. It would indeed be interesting to know the chemical nature of the "crystalloids" since they possess some very unusual regulatory properties.\*

J. P. Martin (1942) induced galls on three-month-old sugar cane plants by injecting extracts obtained from macerated leaf hoppers of the species *Draculacephala mollipes*. These findings again appear to implicate hormone-like substances in the initiation and development of insect galls. Anders (1958) reported that he was able to reproduce the swellings associated with the *Phylloxera* disease by applying to grape plants secretions obtained from the aphid responsible for this condition. A similar type of swelling was obtained when certain amino acids, used in the same proportions found in the insect secretions, were applied to grape roots.

The larva of the moth, *Gnorimoschema gallaesolidaginis*, induces an elliptical monothalmous gall on the stem of the *Solidago* host. The larva in this instance burrows into the terminal bud of the plant and then down the stem to a point 2 cm. below the growing point, where it eats away the central tissues and induces the formation of a simple spindle-shaped gall. The gall stimulus, according to Beck (1954), appears to be associated with a silky substance secreted by the feeding larva. This silky material induces anatomical changes in normal stems similar to those found in the galls. The stimulus is, however, short-lived and uniform and continued deposition of the silky substance over the surface of the larval chamber appears necessary for the formation of typical galls.

Boysen-Jensen (1952) studied, with the use of rather ingenious methods, the development of a midge (*Mikiola fagi*) gall on beech leaves. Evidently the formation of the gall in this instance is caused by growth substances given off by the larva. These as yet uncharacterized substances produce cell enlargement and cell division but not organized growth. It was, therefore, suggested that cell enlargement and cell division are regulated by the larva which moves rapidly about the gall chamber and secretes the growth-promoting substances in definite places, thereby making the gall assume its special form. According to this interpretation, the growth-promoting substance does not have special organizing properties but the shape of the gall is dependent upon the distribution of a rather nonspecific type of growth substance by the larva. The growth substances are, in other words, tools which are used

\* After this paper had been submitted to the editors, a comprehensive account of the studies of Lewis and Walton (1958) appeared. In that investigation the diagnostic crystalloids were found to be Feulgen-positive, and it was concluded that the gall results from the activities of a virus. On the basis of the evidence presented, this conclusion is, in the opinion of the author, unwarranted.



by the gall larva to model a gall from the cells of a beech leaf. While such a mechanism as that proposed by Boysen-Jensen may satisfactorily explain the formation of certain simple galls, it is difficult to see how it would explain the development of the more intricate structures the cells of which are highly differentiated.

The distinguishing feature of insect galls in general, and the more highly developed cynipid galls in particular, is the determinate growth of these structures. Bloch (1954) has suggested that an insect gall is almost comparable—in its “determinate” growth—to a leaf or a fruit. These galls are constant in form and size and possess their own polarity and symmetry. Although the cells of these galls dedifferentiate as a result of the initial stimulus, they again redifferentiate into an orderly rearrangement of cells and cell layers which possess a degree of differentiation that is never below that of the host. A fascinating field lies open here for exploration at the morphogenetic and biochemical levels.

b. *Root Nodules*. The root nodules found to arise on many species of leguminous plants as a result of infection by bacteria of the genus *Rhizobium* are, like the insect galls, highly organized and specialized structures. These nodules, unlike most of the overgrowths described here, are by and large highly beneficial to the host because of the role that they play in nitrogen fixation.

The typical root nodule is composed of four histologically well defined regions. The outermost tissue, or nodular cortex, consists of several layers of parenchymatous cells that originate from the nodular meristem found immediately below the cortex at the distal end of the nodule. The meristem is conspicuous and is composed of small, compact, rapidly dividing cells. The cortex and meristem are normally free of bacteria. The provascular tissue of the nodule arises as a result of radial divisions and differentiation of cells—at the periphery of the inner infected cells and the nodule cortex—at the time when the nodule is still meristematic. These differentiate later into a typical vascular bundle which consists of xylem, phloem fibers, sieve tubes, and companion cells enclosed in parenchymatous tissue and surrounded by an endodermis. The vascular system of the nodule is connected with that of the host and is functional. The central region of the nodule is composed of two types of parenchymatous cells, infected and noninfected, and is commonly known as the bacteroid zone.

Essentially, two types of nodules have been recognized on the basis of their origin. The more common or exogenous type arises, except for the vascular linkages, from the cortical parenchyma of the root. The second or endogenous type is found far less frequently and is composed of cells that arise from proliferation of the pericycle.

The information presented above concerning structure clearly indicates that the nodule is not a shapeless mass of cells but instead represents a highly organized growth. This information, in turn, permits a consideration of the possible mechanisms involved in the growth and development of the nodule.

The infecting bacteria commonly enter the host through root hairs, although in certain aquatic plant species that do not possess root hairs they may and commonly do enter through epidermal cells. Prior to invasion of the host, the bacteria form a small colony close to the tip of the root hair. Under these conditions, the hair curls very characteristically and assumes the form of a "shepherd's crook." As early as 1900, Hiltner found that sterile filtered bacterial extracts, when applied to root hairs, produced a comparable type of deformation. Similar results were reported by Thornton (1936) and Thornton and Nicol (1936) who showed that such filtrates stimulated the production, growth in length, and characteristic deformation of the root hairs. Later, Thornton (1947) found that pure  $\beta$ -indoleacetic acid, when applied to root hairs, elicited a curling similar to that found prior to invasion by the bacteria. Probably, therefore, the characteristic curling of the root hair is the result of the production of an auxin by the bacteria although this point has not yet been fully established. Wilson (1940) points out, for example, that the characteristic curling of the hair is not easily explained on the basis of a growth hormone present in the sterile culture filtrate. He states that one would not expect to find differential growth rates under such experimental conditions since the concentration of the hormone should be the same on all sides of the roots.

After the bacteria enter a hair, they align themselves into hyphalike zoogloal infection thread. The thread penetrates to the base of the hair and then into and directly through the subjacent cortical cells where it branches. As the zoogloal strands migrate through the cortical cells, they become encased in a sheath composed of cellulose-like material, which on the basis of cytochemical studies is said to be composed of the same material as is the wall of the host cell. This has led to the suggestion (McCoy, 1932) that the sheath is deposited by the host cells rather than by the zoogloal strand and that it serves as a defense mechanism against the invading bacteria. Of particular interest to the present discussion is the fact that proliferation of the root cells and subsequent development of the nodule do not occur until the bacteria are released from the infection thread. Release may be achieved essentially in the following ways (Allen and Allen, 1954). The bacteria may be discharged from the tip of the thread as the thread migrates. They may be released as a result of the rupture of unsheathed globular protuberances that are

commonly found to be present in infection threads or they may be liberated following stresses accompanying cell enlargement and cell division. Wipf and Cooper (1940) have suggested, on the basis of cytological studies, that invasion of the disomatic cells by the bacteria is essential for the release of the bacteria from the infection thread as well as for cellular growth terminating in nodule formation.

Once the free bacteria reach the cytoplasm, they tend to migrate to the peripheral regions of the cell and begin to multiply. The host cells containing the organisms, as well as the adjacent uninfected cells, undergo rapid division and subsequent enlargement. Infected cells may increase eightfold in size. Since neighboring uninfected cells are also stimulated to grow, it has been suggested by McCoy (1929) that the bacteria elaborate a diffusible substance which stimulates cell enlargement and cell division. Ultimately, many of the cells in the heart of the nodule become filled with bacteria and the rods found in nodules of certain, but not all, plant species become highly pleomorphic. It is in this so-called bacteroid zone of the nodule that symbiotic nitrogen fixation takes place. This zone also contains four highly interesting pigments, one of which, leghemoglobin, is closely related chemically to blood hemoglobin. The question of the occurrence of the pigments in nodules and their possible role in nitrogen fixation has been reviewed in detail by Allen and Allen (1950, 1954).

Attempts have been made throughout the years to explain in physiological terms the mechanism involved in the development of the nodule. As early as 1912 Molliard reported that he had produced tuberizations on roots of pea plants by treating the roots with cell-free filtrates upon which the rhizobia had grown. It was concluded from these experiments that the bacteria elaborated some biologically active, growth-promoting substance which brought about changes in the root similar to those found during actual infection. Thimann (1936, 1939) examined this question further and postulated the following series of events after the bacteria had penetrated the root. During the course of their metabolism within the host cells the bacteria elaborate a small amount of auxin and certain other substances. The auxin causes an enlargement of the cells in which it is produced but, being diffusible, enters the pericycle and stimulates growth and division, thus giving rise to the early stages of a lateral root initial. In the presence of continued auxin production by the bacteria, the potential lateral root is prevented from elongating. Instead, the cells increase isodiametrically in size. Uninfected cells are stimulated to division by auxin diffusing from the infected cells. In this way, Thimann considers, "a shapeless mass of parenchymatous tissue is produced which is essentially a lateral root prevented from elongating."

Many investigators do not believe, however, that a nodule is simply

a modified lateral root. Fred *et al.* (1932) have discussed the evidence for and against this concept in detail and conclude that the nodule is not a modified lateral root "for it has no central cylinder, root cap, or epidermis. Furthermore, it does not digest its way out from the cortex of the main root but remains covered with a considerable layer of cortical parenchyma."

Nevertheless, growing nodules are active auxin-producing centers and that substance plays an important role in the growth of the nodule (Link and Eggers, 1940). Thimann (1936, 1939) has shown, moreover, that auxin production in the nodule roughly parallels the growth of the nodule.

Chen (1938), Georgi and Beguin (1939), Link (1937), Rasnizina (1938), and Thimann (1939) have demonstrated that *in vitro* the root-nodule bacteria produce considerable quantities of auxin from tryptophan, yeast extract, and peptones present in an otherwise suitable culture medium. Hunt (1951) found, moreover, that free tryptophan was present in the nodules. An evaluation of these findings is complicated, however, by the observation made by Chen (1938) and Georgi and Beguin (1939). These workers found that both effective and ineffective strains of rhizobia produced auxin from tryptophan and in some instances the ineffective strains appeared to be more efficient growth-substance producers than were the effective strains.

While it has been possible to produce overgrowths on the roots of certain leguminous plants by applying growth substances of the auxin type, it nevertheless is true that histologically and anatomically these artificially induced growths do not bear the slightest resemblance to nodules produced under the stimulus of the bacteria. Allen and Allen (1954) point out that the nodule is not a shapeless mass of cells but is a well organized structure. Thus, while there appears to be no conflict on the question of the increased growth-substance content of the nodule, a suitable explanation regarding the role that such a substance plays in directing the organization of the nodule is not yet at hand.

c. *Root Knot*. Root knot, which is caused by species of nematodes belonging to the genus *Meloidogyne*, is a destructive disease in many cultivated and wild plants. The overgrowths produced on the roots may appear as small scattered tubercles or as extensive swellings which may reach diameters up to 2 in. and involve almost the entire root system. Severely infected roots have a rough, clubbed appearance not unlike that encountered in the club root disease of the crucifers. The mature female nematode is embedded in the plant tissue while the eggs are commonly found to be clinging to the sides of the roots. When the eggs hatch, the young larvae may again penetrate young roots just below the growing



point. Once embedded in the tissue, the larva begins to feed and almost at once stimulates the growth of the host cells which leads to the development of the typical knot. The gall, in this instance, is composed of relatively few, greatly hypertrophied cells together with cells showing hyperplasia. Often, 6 to 20 nuclei are present in the enlarged cells although Némec (1910) has reported more than 500 in a single cell of *Vitis gonylodes*. The multinucleate giant cells appear early in gall development. They are rich in storage materials and serve to nourish the larvae in much the same manner as do the "nutritive cell" layers found in certain insect galls. It would appear likely that at least two distinct stimuli, one of which is concerned with cell enlargement and which involves only a few cells and the other with cell division, are involved in the development of this type of overgrowth.

Dropkin (1954) has recently shown a high positive correlation to exist between gall area and numbers of larvae in a gall. He therefore concluded that the response of the root to the presence of the nematode is a local one and that the size of the gall ultimately produced is a function of the amount of stimulation provided by each larva present in the overgrowth. Here, then, is another example of a self-limiting growth, the development of which is dependent upon continued stimulation by the pathogen.

d. *Clubroot*. The typical overgrowth produced on the roots of cabbage by *Plasmidiophora brassicae* is spindle-shaped, thick in the middle, and tapering gradually toward the ends. Such a club is a morphological unit and is commonly the result of a single primary infection. Sometimes, however, the swellings resulting from two or more primary infections fuse to form a compound club which is more irregular in outline than those resulting from a single primary infection. Cunningham (1914) has recognized different types of hypertrophy in different species of susceptible hosts. In cabbage, a complete clubbing of the main roots and of the lateral roots may occur, while in *Sisymbrium altissimum* the lateral roots are commonly free of distortion. In *Sisymbrium officinale* and *Erysimum cheiranthoides*, on the other hand, clubs develop on the lateral roots but not on the main root. Both lateral roots and the main root are affected in *Lepidium sativum*. In this instance, however, club-free rootlets are found above the diseased portions. In the radish, *Raphanus sativus*, clubs occur as tumors on the root.

As a result of the overgrowths, infected roots fail to absorb nutrients from the soil and are often unable to transport food and water taken up by healthy roots. This functional failure of the roots results in the starvation and, ultimately, severe stunting of affected plants. Heavily infected seedlings are usually killed before they reach maturity.

The spread of the disease-producing agent from points of primary infection is accomplished in cabbage through a direct penetration of the host tissue by the infecting plasmodia, which then grows and divides repeatedly. According to Kunkel (1918), infection by penetration may be divided into four parts: (1) primary infection of the cortical tissue and penetration to the cambium, (2) infection of the cambium in all directions from the point of original penetration, (3) passage of the plasmodia out of the cambium into the cortex and in from the cambium toward the xylem region, and (4) infection of the medullary rays.

As the plasmodia pass through the tissues, some of them become established within cells, while others continue to penetrate into the deeper regions. Although no noticeable effect is observed in a cell if infection is temporary, stimulation leading to abnormal cellular growth and division results if the infection of a cell is permanent. The period of infection continues up to the time that the host plant stops growing or dies. The infected cells of a club are distributed in small groups throughout the diseased tissue and do not lie adjacent to each other. The stimulus resulting in abnormal cellular growth appears to travel in advance of the infection. This can be clearly seen when infection is established in the medullary rays. The ray cells—in the proximity of diseased cells—increase considerably in size. This increase in size as well as in number of cells tends to split the central cylinder of an infected root. The mass of parasitic protoplasm in a given volume of diseased tissue is, according to Kunkel (1918), remarkably constant in different clubs. This indicates that the amount of cell growth in this disease, as in the case of root knot, is dependent upon the mass of parasitic protoplasm in the diseased tissue. Clubroot can be considered to be a malignant disease in the sense that this malady may and frequently does kill plants that are affected by it. However, there is no evidence that the affected cells themselves have become permanently altered or that such cells are capable of continued abnormal growth in the absence of the pathogen.

At this point in the discussion we have reached the borderline between the self-limiting growths and the true or non-self-limiting tumors.

### *B. Non-Self-Limiting Tumors*

Tumors of a non-self-limiting and transplantable type may develop in many plant species just as they do in many kinds of animals. In diseases of this type the new growth or tumor is composed of altered, randomly proliferating cells that reproduce true to type and against the growth of which there is no control mechanism in the host. The diseased cells acquire autonomy which permits them to direct their own activities

largely irrespective of the laws that govern very precisely the growth of all normal cells in a higher plant or animal. The nature of this acquired autonomy is fundamental and constitutes the ultimate basis of the tumor problem.

Since the tumor problem is basically a cellular problem and since the fundamental similarity of cells and cellular processes in plants and animals is commonly recognized, certain plant tumors have for many years provided interesting and unique experimental material for studying the metabolic processes that underlie the tumorous state. Certain of the early workers, particularly C. O. Jensen, who is generally considered to be the father of modern experimental cancer research, and Erwin F. Smith, studied on a comparative basis the crown gall disease of plants and malignant animal tumors and they found that these two types of growth have much in common. In comparing plant and animal tumors it must be remembered, however, that there are certain developmental and functional characteristics commonly used in the differentiation of animal tumors that are more or less restricted to animals and cannot, therefore, be carried over and applied to plant tumors. These have been dealt with in detail by White and Braun (1942), and Black (1949) and will not be considered further here. The most essential characteristic of being able to grow independently of any morphogenetic restraint, upon which all other diagnostic features must ultimately depend, is, however, equally capable of expression in neoplasia of all higher organisms since it is a characteristic property of the cell itself.

While it is generally true that the more malignant a tumor cell is the greater is its capacity for growth, it is equally true that a tumor cell is not characterized primarily by its rate of growth. Certain normal cell types may grow and divide at far faster rates than do most tumor cells. Regenerating liver cells, for example, grow much more rapidly than do hepatoma cells present in that organ. Similarly, the meristematic cells at the apex of a rapidly developing root or shoot grow and divide at considerably faster rates than do plant tumor cells. Thus, it is not rapid growth but autonomous growth that characterizes the tumor cell.

Autonomy of neoplastic growth is not, however, a fixed and unvarying character but it has many gradations. At the one extreme are found the slow-growing benign tumors that remain localized in the host. At the other extreme are the most malignant cancers that grow rapidly, infiltrate neighboring tissues, metastasize, and kill. Theoretically, autonomy of tumor cells requires within them something newly activated and distinctive, something that urges such cells to continued abnormal and unregulated growth. One of the difficulties encountered in attempting to define the nature of autonomous growth has resulted from the fact that

some of the most diverse agencies known to biologists are capable of accomplishing this condition. Such factors as radiant energy, irritation, carcinogenic chemicals, microorganisms, and viruses have all been shown to be more or less effective tumor producers in certain animals and plants. The effectiveness of these agencies in eliciting tumor formation appears to be determined in large part by the hereditary constitution of the host. These agencies, with the exception of certain of the viruses, are concerned only with the inception of the tumors and play no role in the continued unregulated growth of a tumor cell. It is therefore necessary to distinguish between the proximate cause or causes concerned in tumor initiation and the continuing cause or causes responsible for tumor development.

Three non-self-limiting diseases of plants, each of which has a different and quite distinct initiating cause, have been studied fairly extensively. These are: (1) the crown gall disease in which an as yet uncharacterized tumor-inducing principle elaborated by a specific bacterium regularly converts normal plant cells to tumor cells, (2) Black's wound tumor disease which is of known viral etiology, and (3) Kostoff's tumors which have a genetical basis and which commonly arise as a result of such a nonspecific stimulus as irritation in certain interspecific hybrids within the genus *Nicotiana*. These are pictured in Fig. 3. There are other true tumorous diseases of plants that have not as yet been extensively studied. Most interesting among these is White's spruce tumor (White and Millington, 1954a, b; Reinert and White, 1956), the etiology of which has not as yet been established.

Although the crown gall, wound tumor, and the genetic tumors have quite different and distinct initiating causes, there is every indication that the ultimate continuing cause is similar at a physiological level in the three types of growth.

The development of an acceptable concept designed to explain the continued abnormal growth of a tumor cell in the absence of any recognizable infective agent (except in the case of the virus-induced tumors) represents a very real challenge to students of abnormal growth. Such a concept must not only explain the underlying basis for autonomous growth but it must also account for the morphological, histological, and cytological peculiarities that characterize the tumorous state.

Plant tumors may assume a variety of growth patterns. These range from slowly growing benign to rapidly growing malignant, and from completely unorganized to highly organized teratomatous growths. All may be produced in certain test systems under precisely defined experimental conditions and all have their counterparts in animal pathology. These are problems of growth and in order to understand them it is





FIG. 3. (A) A primary and a secondary crown gall tumor on a sunflower plant (*Helianthus annuus* L.). (B) Tumors of the type that arise spontaneously in certain interspecific hybrids within the genus *Nicotiana* (*N. langsdorffii*  $\times$  *N. suaveolens*). (C) Crown-gall teratomata on *Kalanchoe daigremontiana*. Note the tumor tissue has a tendency to organize. (D) Black's virus-induced tumor on sweet clover (*Melilotus alba* Desr.). (Photographs by J. A. Carlile.)

necessary to understand something of the processes involved in normal growth and development.

Growth in all animals and plants results either from cell enlargement or from the combined processes of cell division and cell enlargement. These two fundamental growth processes appear to be dependent for their development, in plant cells at least, upon specific substances that may be synthesized by, but are precisely regulated in, all normal plant cells. By varying the ratio of a factor limiting for cell enlargement and one limiting for cell division it is possible to obtain, with the use of certain normal cell types as a test object, either (1) a high degree of organization involving the production of numerous shoots, (2) a completely unorganized callus composed of hypertrophied and hyperplastic cells, or (3) the extensive formation of roots (Skoog and Miller, 1957). The effect of certain limiting factors on the growth of plant cells and on the development of plant organs is clearly evident from studies of this type. It is from this point of view that the three non-self-limiting neoplastic diseases of plants listed above will be discussed.

### 1. *Crown Gall*

The crown gall disease has been studied over the years more intensively than have other plant neoplasms. It has served as the experimental model in the field of plant oncology. Crown gall, which is initiated by a specific bacterium, affects plants belonging to at least 142 genera present in 61 widely separated botanical families. No monocot, however, shows unequivocal response to infection. The bacterium causing this disease possesses the ability to transform normal plant cells to tumor cells in short periods of time. Once the cellular alteration has been completed, the continued abnormal growth of the tumor cell becomes entirely independent of the causal bacteria. The cells of such a tumor continue to grow autonomously at the expense of the host and under favorable conditions the resulting growths may reach enormous size. Crown gall tumors weighing up to 100 lb. have been described in the literature. In certain hosts such as sunflower and Paris daisy there are produced, in addition to primary tumors, secondary tumors that develop at points distant from the seat of the primary inoculation. The secondary tumors are of interest because they are commonly free of the bacteria that initiate the primary growth (Braun, 1941; Smith *et al.*, 1912). This finding permitted the unequivocal demonstration of the truly independent nature of the crown gall tumor cell (Braun and White, 1943; White and Braun, 1942). Tumor cells isolated from such sterile tumors and planted on a suitable culture medium grew profusely and indefinitely on that medium. While the sterile sunflower tumor tissues increased in theo-

retical volume one hundred million million times in the course of one year on that medium, normal cells of the type from which the tumor cells were derived increased in volume only about two hundred and fifty times. This indicates, of course, that a profound change in the metabolism of the affected cells had resulted. Small fragments of sterile tumor tissue when implanted into a healthy host developed again into typical crown gall tumors that were similar in every respect to those originally initiated by the bacteria except that the new growths were free of the inciting organisms. Normal tissue implants, on the other hand, fused with the host and soon fell into the growth pattern of the host without ever forming tumorous overgrowths. Subsequent studies demonstrated that sterile tissue isolated from primary tumors of many different plant species showed the same type of growth autonomy in the host and in culture as did those isolated from the secondary growths (de Ropp, 1947b; Hildebrandt and Riker, 1947, 1949; Morel, 1948; White, 1945).

The commonly found type of crown gall tumor is characterized both in the host and in culture (see Fig. 4, A) by the mostly undifferentiated and completely unorganized growth of its cells. In certain plant species, the cells of which possess highly developed regenerative capacities, there may however be produced, in place of the characteristic crown gall tumor, a complex overgrowth or teratoma which is composed in large part of highly abnormal leaves and buds that show varying degrees of morphological development. Sterile tissues isolated from such organized but abnormal structures grow profusely in culture, as do typical crown gall tumor cells, on a medium that does not support the continued growth of normal callus tissue, as shown in Fig. 4, B and C. Teratoma-derived cells differ from the commonly found crown gall tumor cells, however, in that they retain indefinitely, in culture, a highly developed capacity for organization. The surfaces of such cultures are covered with small, organized structures many of which appear morphologically to be leaves. Histologically, however, these structures are not usually made up of the well differentiated cell types of which leaves are normally composed. An attempt has been made to analyze more precisely the conditions which determine tumor morphology in crown gall (Braun, 1953). Of importance were (1) the strain of the bacteria used to transform normal cells to tumor cells, (2) the relative position that the altered cells occupy in a host, and (3) the inherent competence for regeneration possessed by the affected cells. It was also concluded from this study that the tumor-inducing principle elaborated by highly virulent strains of the bacteria completely overwhelms the cellular factors concerned with differentiation and organization of pluripotent plant cells, while the principle associated with a moderately virulent strain is incapable of com-

pletely suppressing those factors. In the latter instance, the pluripotent teratoma cells retain, despite their conversion to tumor cells, highly developed regenerative capacities. The teratoma tissue is of particular interest because of its usefulness in studying the problem of recovery of crown gall tumor cells (Braun, 1951a).

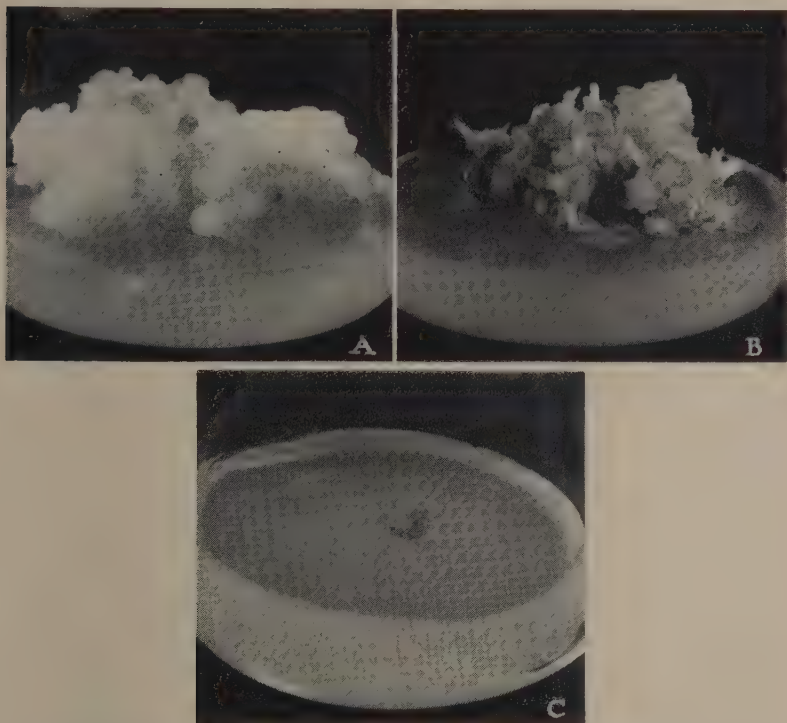


FIG. 4. (A) Crown gall tumor tissue of the unorganized type planted on White's basic medium. (B) Crown gall teratoma tissue. This tissue retains indefinitely a capacity to organize highly abnormal leaves and shoots. (C) Normal tissue of the type from which the tumor tissues were derived. The normal tissue does not grow continuously on this culture medium. (Photographs by J. A. Carlile.)

It is now generally believed that all living nucleated cells of a plant—except perhaps those that are heavily lignified—can be transformed to crown gall tumor cells. Experimental studies have shown, however, that such cells must be conditioned or rendered susceptible to transformation as a result of the stimulus accompanying a wound. It is only during a limited period in the normal wound-healing cycle that normal



plant cells can be converted to tumor cells (Braun, 1952). Following wounding, the host cells gradually become susceptible, reaching a maximum vulnerability between the second and third days after a wound is made. Thereafter, the cells again become progressively more resistant as wound healing progresses toward completion. When a wound is made, the cells in the region of the wound are activated and some two to three days later they begin to divide to heal the wound. Under normal circumstances, upon repair of the wound, the cells which participated in the wound response return again to a quiescent state. In the case of crown gall this return to normalcy is blocked. Some as yet undefined morphogenetic restraint is no longer applied to, or if applied is no longer effective upon, the tumor cells of the regenerating tissues. As a result, the altered cells continue their proliferation in an unregulated and autonomous manner. This unregulated growth results in disorganization of the tissue, in hypertrophy and hyperplasia of the cells, as well as in cytological abnormalities frequently found associated with the tumorous state in crown gall. It is clear, therefore, that the cells proliferating in the tumor are no longer callus cells for they no longer behave as callus cells do. They represent new cell types that have acquired, as a result of bacterial action, new properties the most important of which is the capacity for continued unregulated growth in the absence of any recognizable infective agent.

Any attempt to account in physiological and biochemical terms for the abnormal behavior of a tumor cell must, if it is to receive serious consideration, also account for the morphological, histological, and cytological peculiarities that characterize the tumorous state. Such a concept has been developed and the evidence upon which it is based is outlined briefly below.

Since tumor cells generally appear somehow to have acquired a capacity for autonomous growth as a result of unregulated growth and division, the problem of autonomy has been explored by means of an analysis of the factors concerned specifically with growth accompanied by cell division. As indicated above, the fundamental growth processes of cell enlargement and cell division appear to be dependent for their development in plant cells upon specific substances that may be synthesized by such cells. Crown gall tumor tissue has been shown, moreover, to be a rich source of both a factor normally limiting for cell enlargement and one limiting for cell division. As a result of recent work (Jablonski and Skoog, 1954; Steward and Caplin, 1951), it is now possible to delimit these two growth processes under fully controlled experimental conditions, with the use of certain plant cell types as test objects. When, for example, tobacco pith parenchymal cells are treated with synthetic

growth substances of the auxin type such as naphthalene acetic acid, the pith cells enlarge greatly in size but they do not divide. It is only when a second growth factor such as 6-furfurylaminopurine—or the naturally occurring equivalent of that substance—is supplied to the pith parenchymal cells in addition to an auxin, that a profuse growth accompanied by cell division results. The 6-furfurylaminopurine without an auxin is ineffective in encouraging either an enlargement or a division of the pith cells. These findings demonstrate that two growth substances, one of which is concerned with cell enlargement and the other with cell division, act synergistically to promote growth and cell division in tobacco pith parenchymal cells. Normal tobacco pith cells do not and cannot themselves synthesize these two growth substances for, if they did, they would respond in the characteristic manner indicated above. Since the cellular systems responsible for the synthesis of these two growth substances appear to be solidly blocked in normal tobacco pith cells, a study was undertaken to learn how such cells would respond when transformed to crown gall tumor cells. The results of these studies, which are reported in detail elsewhere (Braun, 1956), demonstrated that when healing pith cells are converted to crown gall tumor cells, typical crown gall tumors develop. This simple experiment demonstrates that, although normal tobacco pith cells are not and cannot synthesize physiologically effective concentrations of either a cell enlargement or a cell division factor prior to their conversion to tumor cells, both substances are synthesized in greater than regulatory amounts following alteration. If this were not true, continued growth accompanied by cell division and, hence, tumor formation would not have resulted in the test system used in these experiments. It is clear, therefore, that an essential difference between a normal tobacco pith cell and a crown gall tumor cell appears to be concerned at a physiological level with the permanent activation of two growth-substances-synthesizing systems the products of which are specifically concerned with growth accompanied by cell division. The continued production in greater than regulatory amounts of the cell enlargement and cell division factors by the tumor cell could account for the continued abnormal proliferation of such a cell. Subsequent studies have shown (Braun, 1957a, b), however, that additional metabolic systems are permanently activated during the transition from a normal cell to a fully altered, rapidly growing type of crown gall tumor cell. It has been found that alteration of normal cells to tumor cells is a gradual but progressive process (Braun, 1943, 1951b). When, for example, the tumor-inducing principle responsible for inception of the crown gall tumor is allowed to act on plant cells for only 34–36 hours before being inactivated by thermal treatment, small, very slowly growing benign

growth are elicited in a host. A 50-hour exposure of cells to the action of that principle results in tumors that grow at a moderately fast rate. If the tumor-inducing principle is allowed to act for 72–96 hours before being destroyed by heat, rapidly growing (potentially malignant) tumors result. It is also possible to obtain tumors showing varying degrees of neoplastic change by allowing the tumor-inducing principle associated with slightly virulent or moderately virulent strains of the crown gall bacteria to act on host cells throughout a 4- or 5-day period. Sterile tissue isolated from the three types of tumors described above and planted on White's basic culture medium retain indefinitely their char-

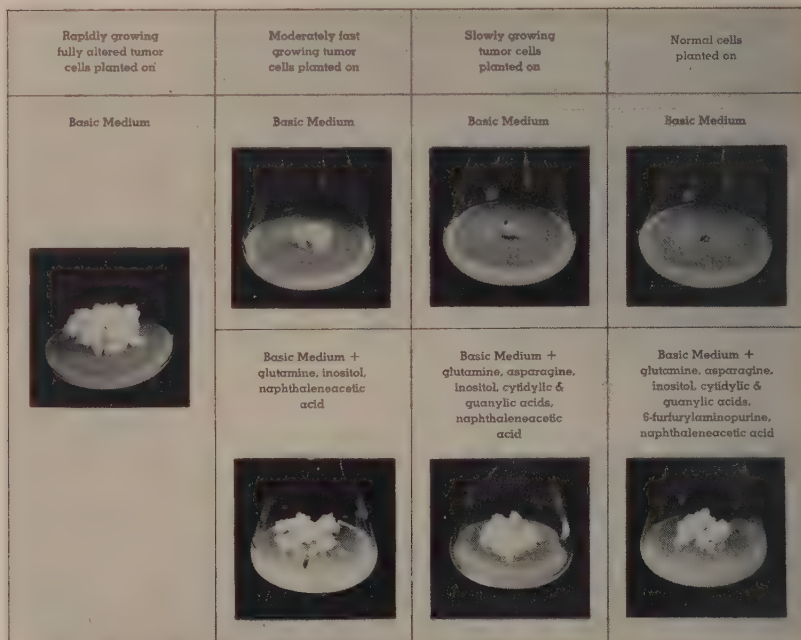


FIG. 5. Relative rates of growth of 3 clones of crown gall tumor tissue that show different degrees of neoplastic change, planted on White's basic medium. (Left) Fully altered, rapidly growing tumor cells. (Upper left) Moderately fast growing tumor cells. (Upper center) Slowly growing tumor cells. (Upper right) Normal cells of the type from which the tumor cells were derived. While the 3 clones of the tumor cells grow continuously, although at different rates on the basic culture medium, normal cells of this type do not grow on that medium. Lower pictures and legends show minimal nutritional supplements needed by the 3 types of tissues to achieve a growth rate comparable to that of the fully altered tumor cell. (Photographs by J. A. Carlile.)

acteristic growth patterns. This is illustrated in Fig. 5. Since these three types of tumors are derived from the same plant species, they are admirably suited for a study of the factors required for rapid autonomous growth. In these studies, the results of which are summarized in Fig. 5, the rapidly growing fully altered tumor cell was used as the standard. This cell type can synthesize in optimal or near optimal amounts all of the growth factors needed for its continued rapid abnormal growth from mineral salts and sucrose present in White's basic culture medium. The moderately fast growing tumor cell required that the basic medium be supplemented with glutamine, meso-inositol, and a cell enlargement factor (naphthalene acetic acid) to achieve a growth rate comparable to that of the fully altered rapidly growing type of tumor cell. The very slowly growing benign tumor cells altered in a 34-hour period required, in addition to the three compounds described above, asparagine as well as cytidylic and guanylic acids to achieve full growth. It is clear from experiments of this type that, as the crown gall tumor cell becomes more autonomous, its requirements in terms of externally supplied growth factors become less exacting. These studies demonstrate clearly, moreover, that a series of well defined growth-substance-synthesizing systems become gradually activated during the transition from the normal cell to the fully altered tumor cell, and the degree of activation of these systems determines the rate of growth of the tumor cell.

Normal cells of the type from which the tumor cells are derived do not grow on the basic medium. Thus, although the difference between the three types of tumor cells is quantitative since all grow continuously, though at different rates, on the basic medium, the difference between the tumor cells and the normal cell is qualitative. One qualitative difference found to exist in these studies is the absolute requirement of the normal cell for 6-furfurylaminopurine or the naturally occurring equivalent of that substance. The addition of that compound to the basic media or to the supplemented culture media did not stimulate growth of any of the tumor tissues. The normal cells also possess, in contrast with the tumor cells, an absolute requirement as a supplement for a cell enlargement factor such as naphthalene acetic acid. The addition of 6-furfurylaminopurine and naphthalene acetic acid to the basic medium permits the very slow growth of normal cells. However, only if the basic medium is supplemented with glutamine, asparagine, inositol, guanylic and cytidylic acids, in addition to the auxin and 6-furfurylaminopurine, do the normal cells achieve a growth rate comparable to that of the fully altered, rapidly growing type of tumor cell. It thus appears that, as a result of the transition from a normal cell to a fully altered, rapidly



growing crown gall tumor cell, a series of quite distinct but well defined growth-substance-synthesizing systems become progressively activated. This leads to the production by the affected cell of greater than regulatory amounts of these growth-promoting substances. The continued production in greater than regulatory amounts of these substances by the tumor cell could and most probably does account for the continued unregulated proliferation of such a cell. Precisely how the tumor-inducing principle associated with this disease accomplishes the simultaneous unblocking of several apparently distinct and quite unrelated metabolic systems remains unanswered. These results are nevertheless understandable if it is assumed that some as yet uncharacterized master reaction within the cell is specifically but gradually unblocked by the tumor-inducing principle and which, once activated, not only accomplishes the unblocking of several other growth-substance-synthesizing systems but also determines the rate at which the entire series of metabolic events concerned with growth and cell division proceeds.

The concept of growth autonomy presented above finds additional support in other directions. It has been possible to reproduce, under precisely defined experimental conditions and with the use of certain normal cell types as a test object, not only the morphological growth patterns (Braun and Näf, 1954) (slow and rapid disorganized growths, teratoma-like structures) but also the histological (hypertrophy and hyperplasia leading to disorganization and loss of function) as well as the cytological (multinucleate giant cells, etc.) (Skoog, 1954) abnormalities that characterize the tumorous state in crown gall. This was accomplished by varying the proportions of the cell enlargement factor and the factor limiting for cell division in an otherwise suitable culture medium on which the normal cells were planted. These artificially stimulated normal cells, in contrast to crown gall tumor cells, are self-limiting growths and when the externally supplied stimuli are removed, their growth promptly stops. The fact that such stimulated normal cells commonly show histological and cytological characteristics of true tumor cells but are themselves self-limiting growths indicates that the observed cellular abnormalities are the result rather than the cause of the tumorous state.

Quite possibly, a cell acquires the capacity for autonomous growth as a result of the permanent activation of a series of growth-substance-synthesizing systems the products of which are concerned specifically with growth accompanied by cell division. These systems are precisely regulated in all normal plant cells.

Here, then, is a rare example of a disease in which an infective agent

in some as yet uncharacterized manner regularly converts normal plant cells into new cell types in short periods of time. Once the cellular transformation has been accomplished, the pathogen no longer plays a role in the continued development of the disease. The altered host cells become pathogenic by virtue of the fact that those cells have acquired a capacity to synthesize greater than regulatory amounts of growth-promoting substances of a type that are limiting for growth and cell division in normal plant cells.

Although growth-promoting substances elaborated by the tumor cells themselves appear to be responsible for the continued unregulated growth of such cells, other sources of growth hormone may influence the morphological growth patterns as well as the rates of growth of the resulting tumors. This is particularly evident when dealing with crown gall tumor cells that grow slowly and possess relatively low grades of neoplastic change. For example, the tumor-inducing principle elaborated by certain attenuated strains of the crown gall bacteria initiates very slowly growing tumors on plants such as the tomato. The application of synthetic hormones of the auxin type to such normally slowly growing tumors results in the formation of large rapidly growing tumors (Braun and Laskaris, 1942). In this instance, the effect of the growth-promoting substance on the cell is only temporary and, although growth is enhanced, the increased growth rate does not become an intrinsic property of the cell itself. Not only the rate of growth but also the tendency of tumor tissue to organize may be hormonally influenced. When, for example, the tumor-inducing principle elaborated by a moderately virulent strain of the bacterial pathogen transforms pluripotent cells at the cut stem tip of a tobacco plant, complex tumors or teratomata arise. When, on the other hand, this same tumor-inducing principle alters similar cells in an internode of a plant containing a functional apical bud, and hence hormone-producing center, typical unorganized crown gall tumors develop. In this instance, the functional apical bud can be wholly replaced in suppressing the tendency of the tumor tissue to organize by synthetic growth hormones. The effect of the hormone in suppressing organization in the tumor tissues is only temporary, as evidenced by the fact that tissue isolated from either the organized teratomata or unorganized tumors, when planted on a culture medium, shows identical growth patterns. In both instances, the tissues are characterized by a capacity to organize highly abnormal shoots and buds. It can, therefore, be concluded that the greater the degree of the primary change in a tumor cell and, hence, the greater the growth-substance output by that cell, the less effective are externally supplied hormones in stimulating growth.

The growth of fully altered, rapidly growing crown gall tumor cells is, in fact, inhibited when such cells are treated with growth substances of the auxin type.

## 2. Wound Tumor

A second plant disease which fulfills all of the criteria of a true neoplastic disease is one caused by a typical virus. This malady is known as the wound tumor disease. The etiological entity responsible for this disease is insect-transmitted and is one of a group of viruses that multiply in the insect as well as in the plant host.

On a large variety of plants (Black, 1949, 1952, 1954), the symptoms produced in response to the virus involve morphogenetic disturbances. A survey of potential hosts indicated that at least 43 species belonging to 20 plant families produce symptoms typical of the disease, including irregular enlargements of the veins in leaves and tumors on the roots. Other symptoms observed included leaf curling and distortion, leafy outgrowths from the undersides of the veins, vein tumors, distortion of petioles, and shortening of internodes with a resulting dwarfing of the infected plant.

The variations in symptoms vary from barely detectable to those that are extremely pronounced. In *Portulaca* the tops of the infected plants bear no symptoms, but the roots contain many small tumors. In some plants such as sorrel and sweet clover the response to infection involves the production of large tumors which in the case of sweet clover appear on the stem. Investigations on the latter host demonstrated that the hereditary constitution of the plant affects the number, size, distribution, and morphology of the tumors (Black, 1951). In some strains the root tumors may be so large and numerous that they fuse together, while in others so inconspicuous that they may easily be overlooked. Similarly, in some clones, stem reaction involves the formation of many large tumors; in others they are rarely found. Moreover, hereditary influences on the stem and root may act independently. It has been found, for example, that one clone produces large tumors on stems and roots while another produces many large tumors on the roots but only occasional tumors on the stem.

That the genetic basis which causes a clone to be highly predisposed to tumor formation involves not merely a susceptibility to the virus but also an inherent tendency toward tumorous proliferation is indicated by the findings of Littau and Black (1952). These investigators found that the inbred B21 clone of sweet clover, which responds actively with tumor formation as a result of virus infection, produced five spontaneous tumors over a period of several years. That these new growths were not the

result of accidental virus infection was adequately demonstrated. The authors have compared their B21 line of sweet clover with strain C<sub>3</sub>Hb of mice which have lost the mammary carcinoma virus and yet show a strong inherent tendency toward the development of mammary carcinoma.

In this, as in other neoplastic diseases of plants, wounding plays an important role in the initiation of the disease. Tumors develop from accidental or artificially made wounds, from points of stress, as well as in those regions where lateral roots emerge. Black and Lee (1957) have also demonstrated that when infected plants are treated with an auxin, a pronounced increase in the number and size of stem tumors results. Thus, at least three factors appear to be essential for the initiation and development of tumors in this disease: (1) the inciting virus, (2) a wound or some similar stimulus, and (3) the hereditary constitution of the host.

The function of the wound is not yet clear. Brakke *et al.* (1954) have suggested that perhaps the response of a cell to a wound permits an increase in virus concentration and that the high concentration maintains the cells in a state of active proliferation. The cells present in tumors of sweet clover have been found to contain about one hundred times more virus than do nontumorous portions of a diseased plant. Another possibility presents itself, however. It may be that virus-infected and hence potential tumor cells do not develop into a neoplastic growth until they are first stimulated to divide by noncarcinogenic processes such as wounding, application of hormones, etc. Once cell division is initiated as a result of a nonspecific stimulus, the infected cells are no longer subject to the morphogenetic restraints in a host which normally return the cell to quiescence after wound healing has gone to completion. Kelly and Black (1949) have raised the very pertinent question as to why one cell in the pericycle of an infected sweet clover root develops into an organized lateral root, while other pericycle cells in close proximity develop into disorganized tumors. There are several possible answers to this question. Since lateral root formation occurs earlier than tumor formation, it may be that the more embryonic tissue has such tight restraints placed upon it that the infection does not lead to its disorganization. On the other hand, lateral roots, initially at least, appear to involve the xylary pericycle while the tumors generally involve the phloic pericycle. These differences may reflect divergencies in morphogenetic response at the two sites. A third possibility rests on the assumption that only certain cell types are infected by the virus. The leaf hopper-transmitted viruses, of which the wound tumor virus is a representative, appear to show a predilection for phloem tissue. Since lateral root forma-



tion initially involves the xylary pericycle, it may be that at the stage of root development that permits lateral root initiation, only that portion of the pericycle closest to the phloem is infected with virus. It would, therefore, not be until lateral root formation involved tissue close to the phloem that a tumor would develop, because not until then would the cell division stimulus associated with lateral root formation involve virus-infected cells.

Once tumors are initiated, the virus remains closely associated with the tumorous tissue. These tumors possess a capacity for indefinite growth, both in the host and in culture. Sterile tumor tissue isolated from sorrel and planted on White's basic culture medium initially grew rather slowly and doubled its volume every 3 weeks. Subsequently, Burkholder and Nickell (1949) devised a more suitable culture medium which permitted far more rapid growth of the tumor tissue. The tumor tissue in culture was found to possess an unusually high requirement for phosphorus. This was interpreted to reflect the need of the multiplying virus for this substance. Similarly, an RNA hydrolyzate, and more specifically uracil, was found to exert a stimulatory effect on the growth of the tumor tissues.

There is no question about the fact that specific viruses are etiologically implicated in the wound tumor disease of plants as well as in certain virus-induced tumors of animals. The question that arises, however, is: How does the virus induce the neoplastic state in a host cell? Viruses cause cells of plants and animals to respond to infection in many different ways. Few elicit tumorous growths. Even the tumor-inducing viruses may infect cells and not exert a stimulatory influence on such cells. What is it then that causes certain viruses to induce cells to proliferate in an essentially unregulated and uncontrolled manner? There is a partial answer at least in the case of Black's wound tumor disease. The virus-infected tissue, like crown gall tumor tissue, acquires a capacity to synthesize growth-promoting substances concerned specifically with growth and cell division. This is evidenced by the fact that the tumor cells grow rapidly and indefinitely on a culture medium that is lacking in certain growth factors needed for the continued growth of normal cells. It thus appears that the presence of the virus confers upon the cell the ability to produce such growth-promoting substances in greater than regulatory amounts and as a result the cell achieves a physiological autonomy.

### 3. *Genetic Tumors*

The formation of non-self-limiting tumors, in the development of which no demonstrable infectious agent is involved, is a regular occur-

rence in certain plants. In these instances, it is the genetic constitution of the cells within a plant that appears to be of importance. The most thoroughly investigated examples of this type involve the development of spontaneously occurring tumors in a large number of *Nicotiana* hybrids. Kostoff (1930) demonstrated that when appropriate *Nicotiana* species are crossed, tumors develop in all hybrid offspring. These hybrid plants are interesting because they are, for the most part, perfectly organized both morphologically and histologically during their period of active growth and in the absence of irritation. Once such plants reach maturity and terminal growth ceases, a profusion of tumors may develop on all parts of the plants. It is, however, possible, according to Kunkel (1954), to initiate a tumor at almost any period in the development of a plant by making a wound in the vicinity of a vascular bundle. The cells of such plants behave as do normal cells until they are stimulated to divide. Once the cells of this plant are activated as a result either of natural processes or artificially induced irritation, they no longer respond to the morphogenetic restraints that return a normal cell to quiescence. The genetic constitution of the cell, in this instance, is critical. Only such a nonspecific stimulus, such as irritation, is required to transform the potential tumor cells, of which the hybrid plant is composed, into actively proliferating autonomous plant-cell types.

Since Kostoff's original description of this phenomenon, the number of tumor-producing combinations has increased considerably (Kehr, 1951; Kehr and Smith, 1954; Näf, 1958). The tumors apparently arise in tissue that has been stimulated to division by natural or artificial processes and, once initiated, the tumors retain the capacity for unlimited disorganized growth both *in vitro* and *in situ*. Normally, tumors on the stem develop at the site of leaf or petal abscission. A number of hybrid combinations have been found to produce tumors on the roots only. In those many instances in which tumors develop on all parts of the plant, tumor formation on the root often precedes the formation of overgrowths on the shoot. This increased response on the part of the root may result for several reasons. Lateral root formation may represent a stimulus similar to wounding and may, therefore, play a role in tumor initiation similar to that found in the virus tumors. Irradiation of hybrid plants hastens the onset of tumor formation and increases significantly the number of tumors that develop (Sparrow *et al.*, 1956). Chemical irritation may also initiate tumors. Kehr and Smith (1954) reported that leaves of certain hybrid combinations accidentally sprayed with a mixture of turpentine, whiting, and white lead, produced tumors at almost every spot where droplets of the spray mixture fell on a leaf.

The genetic tumors appear to represent a lower grade of neoplastic

change than do most crown gall tumors. This is evidenced both by the fact that the stem tumors show a strong tendency to organize abnormal structures and that they seldom, if ever, reach the size and state of disorganization of crown gall tumors initiated by highly virulent bacteria. Although these tumors are almost never directly fatal to the plant, they do represent a considerable burden to the plant.

Kehr and Smith (1952, 1954) attempted to analyze the precise genetic basis of these tumors by breeding a considerable number of diploid and polyploid combinations of *Nicotiana glauca*  $\times$  *N. langsdorffii*. From the data obtained it was concluded that the tumor-forming nature of the hybrid remains relatively unchanged regardless of the ratio of *N. glauca* and *N. langsdorffii* genomes as long as at least one complete genome of each species is present in the hybrid. It was found, further, that, although spontaneous genetic tumors develop when all twelve *N. glauca* chromosomes are combined with a diploid complement of *N. langsdorffii* chromosomes, no tumors developed in hybrid plants when only one or a few *N. glauca* chromosomes are present in addition to the diploid *N. langsdorffii* genome.

Recently Näf (1958) has approached the problem from a different point of view. This worker has divided all of the parents of tumorous hybrids into two groups which he arbitrarily designated as "plus" and "minus." Näf found that if an intragroup cross is made either between two "plus" species or two "minus" species, the offspring never develops tumors. On the other hand, crosses made between a "plus" species and a "minus" species produce tumorous offspring. Of a total of more than 50 crosses tested, very few exceptions to the above rule were found. Näf explains these exceptions on the basis of relative "plusness" and "minusness," similar to Hartmann's concept of relative sexuality. It was concluded from these studies that the critical contributions to tumor formation of the "minus" parents differ from those of the "plus" parents. These contributions, since they are of a genetical nature, must be reflected in parental metabolism and it should be possible to characterize them on a physiological level.

As in the case of crown gall and Black's virus tumor, tissue isolated from the hybrid tumors and planted in culture is capable of synthesizing all of the growth factors required for its continued abnormal growth from mineral salts and sucrose present in a basic culture medium. What is particularly interesting in the case of the hybrid is that cells obtained from nontumorous portions of hybrid stems also become autonomous upon isolation and culture. Such tissue is truly tumorous and, as White (1944) has shown, it can be grafted to one of the parents, *N. glauca*, where the implants again develop into tumorous overgrowths. Hybrid

tumor tissue does not commonly differentiate and organize when grown on a semisolid medium. White (1939) found, however, that when such tissue is immersed in a liquid medium, it tends to organize shoots and leaves. It was presumed that oxygen gradients influenced the differentiation process. Skoog (1944) found, however, that the tendency to form buds and leaves can be completely suppressed by the addition of 0.2 p.p.m. of indoleacetic acid. Low concentrations of indoleacetic acid were found not only to suppress organization but also to stimulate growth. The indoleacetic acid effect was reversible by raising the level of certain nutrients such as sucrose,  $\text{KH}_2\text{PO}_4$ , and  $\text{Fe}_2(\text{SO}_4)_3$ . Subsequent studies on tobacco (Skoog and Tsui, 1951; Miller and Skoog, 1953) demonstrated that bud formation on isolated stem segments of tobacco depends upon an adenine:indoleacetic acid ratio. Adenine favors bud development which, in turn, may be inhibited by the addition of indoleacetic acid. More recently, Skoog and Miller (1957) have reported that 6-furfurylaminopurine is far more effective than adenine, in this respect.

The capacity of hybrid tumor tissue to organize shoots and buds under certain conditions does not reflect a tendency of such tissue to return to normalcy since these organized structures are composed entirely of potential tumor cells and they would appear, therefore, to be organized tumors.

The following picture emerges from studies on the non-self-limiting neoplastic diseases of plants. It is apparent from this discussion that several quite distinct agencies can bring about the tumorous state. The biological aspects of this state are characterized by growth autonomy. This phenomenon can be adequately explained on the basis of the ability of the tumor cell to synthesize certain growth-promoting substances in greater than regulatory amounts. The rate of growth of a tumor cell appears, moreover, to be a function of the degree to which the growth-substance-synthesizing systems within a cell are activated. The ultimate mechanism by which the growth-substance-synthesizing systems become activated in plant tumor cells is unknown. The production of these substances by the cell is, however, presumably enzymatic in nature.

Since enzymatic reactions are commonly considered to be gene controlled processes, it might appear that the normal gene complement is somehow modified in the plant tumor cell. This could conceivably be accomplished by somatic mutation at the genic level. There are several reasons for questioning somatic mutation as being the cause of the physiological autonomy that underlies the tumorous state in plants. The first of these is that a specific virus has been implicated etiologically in one of the diseases in question. It might be argued, however, that the virus induces a mutation at the genic level in cells and that once this



change is effected, the virus is no longer needed for the continued abnormal growth of the tumor cell. While this possibility cannot at present be ruled out in the case of Black's virus tumor, other closely related viruses that produce abnormal growth patterns in plants have been eliminated from their hosts by thermal treatment with a resulting complete recovery of such plants (Kunkel, 1936, 1941). Plant viruses of this type do not appear, therefore, to induce permanent modifications in plant cells. The continued abnormal behavior of the cell seems to be dependent upon the continued presence of the virus. In this respect, the virus-induced tumors behave as does the clubroot disease of the crucifers in which continued cell stimulation is dependent upon the continued presence of the pathogen.

The reported recovery of crown gall tumor cells also appears to argue against somatic mutation as the cause of the physiological autonomy found in this type of tumor cell. By forcing abnormal tumor buds present in crown gall teratomata into very rapid growth by a series of graftings to healthy plants, a gradual but ultimately complete recovery of such cells was achieved. These findings suggest that crown gall tumor cells may recover if they are forced to divide with unusual rapidity at the stem apex. They suggest further that the factor responsible for the continued abnormal proliferation of the crown gall tumor cell is an autonomous or partially autonomous entity that is subject to the effects of dilution in cells that are forced to divide with great rapidity (Braun, 1954).

The findings reported above are very suggestive of some encountered in microbial genetics. Studies such as those presented by Ephrussi (1951), Sonneborn (1946), and Spiegelman (1954) indicate that certain self-duplicating cytoplasmic factors as well as the nuclear genes may serve as determinants of hereditary differences in a cell. Certain of these cytoplasmic entities appear, moreover, to be concerned with enzyme production. Therefore, mechanisms quite different from somatic mutation at the gene level can be postulated to explain the continuity of tumorous properties from one cell generation to the next. It may well be that it is in the particulate cytoplasmic fraction of the cell rather than in the nucleus that changes occur which account for the physiological autonomy that underlies the tumorous state in plants.

#### ACKNOWLEDGMENTS

Figures 3, A, B, D, and Figure 5 were previously published in an article entitled "The Morphology and Physiology of Plant Tumors," by Armin C. Braun and T. T. Stonier, in *Protoplasmatologia*, 1958.

The author wishes to acknowledge his indebtedness to Miss Ella J. Ross for invaluable assistance in the preparation of the manuscript.

## REFERENCES

- Allen, E. K., and O. N. Allen. 1950. Biochemical and symbiotic properties of the rhizobia. *Bacteriol. Rev.* **14**: 273-330.
- Allen, O. N., and E. K. Allen. 1954. Morphogenesis of the leguminous root nodule. *Brookhaven Symposia in Biol.* **No. 6**: 209-234.
- Anders, F. 1958. Aminosäuren als gallenerregende Stoffe der Reblaus (*Viteus [Phylloxera] vitifolii* Shimer). *Experientia* **14**: 62-63.
- Beck, E. G. 1954. The nature of the stimulus in the "Solidago" gall induced by the larva of "Gnorimoschema gallaesolidaginis." *Brookhaven Symposia in Biol.* **No. 6**: 235-251.
- Beijerinck, M. W. 1888. Ueber das Cecidium von *Nematus capreae* auf *Salix amygdalina*. *Botan. Zeitung* **46**: 1-28.
- Beijerinck, M. W. 1897. "Verzammelte Geschriften," Vol. 3 especially page 203.
- Black, L. M. 1949. Virus tumors. *Survey Biol. Progr.* **1**: 155-231.
- Black, L. M. 1951. Hereditary variation in the reaction of sweet clover to the wound-tumor virus. *Am. J. Botany* **38**: 256-267.
- Black, L. M. 1952. Plant virus tumors. *Ann. N. Y. Acad. Sci.* **54**: 1067-1075.
- Black, L. M. 1954. Plant tumor diseases and the relation of some of them to viruses. *Proc. Natl. Cancer Conf. 2nd Conf. 1954.* **2**: 1349-1355.
- Black, L. M., and C. L. Lee. 1957. Interaction of growth-regulating chemicals and tumefacient virus on plant cells. *Virology* **3**: 146-159.
- Bloch, R. 1938. Anatomical changes in *Tradescantia fluminensis* Vell. after treatment with growth substances. *Contrib. Boyce Thompson Inst.* **9**: 439-454.
- Bloch, R. 1954. Abnormal plant growth. *Brookhaven Symposia in Biol.* **No. 6**: 41-54.
- Bos, L. 1957. Heksenbezemverschijnselen een pathologisch-morfologisch onderzoek. *Mededel. Landbouwhogeschool Wageningen* **57**: 1-79.
- Boysen-Jensen, P. 1952. Untersuchungen über die Bildung der Galle von *Mikiola fagi*. *Kgl. Danske Videnskab. Selskab. Biol. Medd.* **18**(18): 18 pp.
- Brakke, M. K., A. E. Vatter, and L. M. Black. 1954. Size and shape of wound-tumor virus. *Brookhaven Symposia in Biol.* **6**: 137-156.
- Braun, A. C. 1941. Development of secondary tumors and tumor strands in the crown gall of sunflowers. *Phytopathology* **31**: 135-149.
- Braun, A. C. 1943. Studies on tumor inception in the crown-gall disease. *Am. J. Botany* **30**: 674-677.
- Braun, A. C. 1951a. Recovery of crown-gall tumor cells. *Cancer Research* **11**: 839-844.
- Braun, A. C. 1951b. Cellular autonomy in crown gall. *Phytopathology* **41**: 963-966.
- Braun, A. C. 1952. Conditioning of the host cell as a factor in the transformation process in crown gall. *Growth* **16**: 65-74.
- Braun, A. C. 1953. Bacterial and host factors concerned in determining tumor morphology in crown gall. *Botan. Gaz.* **114**: 363-371.
- Braun, A. C. 1954. Studies on the origin of the crown-gall tumor cell. *Brookhaven Symposia in Biol.* **6**: 115-127.
- Braun, A. C. 1956. The activation of two growth-substance systems accompanying the conversion of normal to tumor cells in crown gall. *Cancer Research* **16**: 53-56.
- Braun, A. C. 1957a. A physiological study on the nature of autonomous growth in neoplastic plant cells. *Symposia Soc. Exptl. Biol.*, **No. 11**: 132-142.
- Braun, A. C. 1957b. Tissue culture as a tool for studying the development of autonomy in neoplastic plant cells. *J. Natl. Cancer Inst.* **19**: 753-769.

- Braun, A. C., and T. Laskaris. 1942. Tumor formation by attenuated crown-gall bacteria in the presence of growth-promoting substances. *Proc. Natl. Acad. Sci. U. S.* **28**: 468-477.
- Braun, A. C., and U. Näf. 1954. A non-auxinic growth-promoting factor present in crown gall tumor tissue. *Proc. Soc. Exptl. Biol. Med.* **86**: 212-214.
- Braun, A. C., and P. R. White. 1943. Bacteriological sterility of tissues derived from secondary crown gall tumors. *Phytopathology* **33**: 85-100.
- Brian, P. W. 1957. The effects of some microbial metabolic products on plant growth. *Symposia Soc. Exptl. Biol.*, No. **11**: 166-182.
- Brian, P. W. 1958. Role of gibberellin-like hormones in regulation of plant growth and flowering. *Nature* **181**: 1122-1123.
- Brian, P. W., and H. G. Hemming. 1955. The effect of gibberellic acid on shoot growth of pea seedlings. *Physiol. Plantarum* **8**: 669-681.
- Brown, N. A., and F. E. Gardner. 1936. Galls produced by plant hormones, including a hormone extracted from *Bacterium tumefaciens*. *Phytopathology* **26**: 708-713.
- Bryan, M. K. 1915. A Nasturtium wilt caused by *Bacterium solanacearum*. *J. Agr. Research* **4**: 451-458.
- Burkholder, P. R., and L. G. Nickell. 1949. Atypical growth of plants. I. Cultivation of virus tumors of *Rumex* on nutrient agar. *Botan. Gaz.* **110**: 426-437.
- Chen, H. K. 1938. Production of growth-substance by clover nodule bacteria. *Nature* **142**: 753-754.
- Cross, B. E., J. F. Grove, J. MacMillan, and T. P. C. Mulholland. 1956. Gibberellic acid. IV. Structures of gibberic and allogibberic acids and possible structures of gibberellic acid. *Chem. & Ind. (London)*. **1956**: 954-955.
- Cunningham, G. E. 1914. Studies on club root. II. Disease resistance of crucifers; methods of combating club root. *Vermont Univ. Agr. Expt. Sta. Bull.* **185**: 65-96.
- de Ropp, R. S. 1947a. The growth-promoting and tumefacient factors of bacteria-free crown-gall tumor tissue. *Am. J. Botany* **34**: 248-261.
- de Ropp, R. S. 1947b. The isolation and behavior of bacteria-free crown-gall tissue from primary galls of *Helianthus annuus*. *Phytopathology* **37**: 201-206.
- de Vries, H. 1909-1910. "The Mutation Theory," Open Court Pub. Co., Chicago. 2 Vols.
- Dimond, A. E., and P. E. Waggoner. 1953. The cause of epinastic symptoms in *Fusarium* wilt of tomatoes. *Phytopathology* **43**: 663-669.
- Dropkin, V. H. 1954. Infectivity and gall size in tomato and cucumber seedlings infected with *Meloidogyne incognita* var. *acrita* (root-knot nematode). *Phytopathology* **44**: 43-49.
- Ephrussi, B. 1951. Remarks on cell heredity. In "Genetics in the 20th Century" (L. C. Dunn, ed.). Macmillan, New York. pp. 241-262.
- Felt, E. P. 1940. "Plant Galls and Gall Makers." Comstock, Ithaca, New York. 364 pp.
- Fred, E. B., I. L. Baldwin, and E. McCoy. 1932. Root nodule bacteria and leguminous plants. *Univ. Wisconsin Studies in Sci.* No. **5**: 343 pp.
- Gäumann, E. 1946. "Pflanzliche Infektionslehre." Birkhäuser, Basel. 611 pp.
- Gäumann, E. 1954. Toxins and plant diseases. *Endeavour* **13**: 198-204.
- Georgi, C. E., and A. E. Beguin. 1939. Heteroauxin production by efficient and inefficient strains of *Rhizobia*. *Nature* **143**: 25.
- Grieve, B. J. 1936. Effect of inoculation of plant stems with *Bacterium solanacearum*. *Nature* **137**: 536.

- Grieve, B. J. 1939. Epinastic response induced in plants by *Bacterium solanacearum* E. F. S. *Ann. Botany (London)* **3**: 587-600.
- Grieve, B. J. 1940. Studies in the physiology of host-parasite relations. II. Adventitious root formation. *Proc. Roy. Soc. Victoria* [N.S.] **53**: 323-341.
- Grieve, B. J. 1941. Further observations on rose wilt virus. *Proc. Roy. Soc. Victoria* [N.S.] **54**: 229-238.
- Grieve, B. J. 1943. Mechanism of abnormal and pathological growth: A review. *Proc. Roy. Soc. Victoria* [N.S.] **55**: 109-132.
- Harvey, R. B. 1918. Hardening process in plants and developments from frost injury. *J. Agr. Research* **15**: 83-112.
- Hayashi, T., Y. Takijima, and Y. Murakami. 1953. Biochemical studies of bakanae fungus. XXVIII. The physiological action of gibberellin. *J. Agr. Chem. Soc. Japan* **27**: 672-675. (*Chem. Abstr.* **48**: 12920, 1954).
- Heinricher, E. 1915. Zur Frage nach der assimilatorischen Leistungsfähigkeit der Hexenbesen des Kirschbaumes. *Ber. deut. botan. Ges.* **33**: 245-253.
- Heslop-Harrison, J. 1952. A reconsideration of plant teratology. *Phyton, Ann. rei botan.* **4**: 19-34.
- Hildebrandt, A. C., and A. J. Riker. 1947. Influence of some growth-regulating substances on sunflower and tobacco tissue *in vitro*. *Am. J. Botany* **34**: 421-427.
- Hildebrandt, A. C., and A. J. Riker. 1949. The influence of various carbon compounds on the growth of marigold, Paris-daisy, periwinkle, sunflower and tobacco tissue *in vitro*. *Am. J. Botany* **36**: 74-85.
- Hiltner, L. 1900. Ueber die Ursachen, welche die Grösse, Zahl, Stellung und Wirkung der Wurzelknöllchen der Leguminosen bedingen. *Arb. biol. Reichsanst. Land- u. Forstwirtschaft, Berlin-Dahlem* **1**: 177-222.
- Hunger, F. W. T. 1901. Een Bakterie-ziekte der Tomaat. *Mededeel. uit's Lands Plantentuin, Batavia* **48**(4).
- Hunt, G. E. 1951. A comparative chromatographic survey of the amino acids in five species of legume roots and nodules. *Am. J. Botany* **38**: 452-457.
- Jablonski, J. R., and F. Skoog. 1954. Cell enlargement and cell division in excised tobacco pith tissue. *Physiol. Plantarum* **7**: 16-24.
- Jones, D. F. 1935. The similarity between fasciations in plants and tumors in animals and their genetic basis. *Science* **81**: 75-76.
- Kehr, A. E. 1951. Genetic tumors in *Nicotiana*. *Am. Naturalist* **85**: 51-64.
- Kehr, A. E., and H. H. Smith. 1952. Multiple genome relationships in *Nicotiana*. *Cornell Univ. Agr. Exptl. Sta. Mem.* **311**: 1-19.
- Kehr, A. E., and H. H. Smith. 1954. Genetic tumors in *Nicotiana* hybrids. *Brookhaven Symposia in Biol.* **6**: 55-78.
- Kelly, S. M., and L. M. Black. 1949. The origin, development and cell structure of a virus tumor in plants. *Am. J. Botany* **36**: 65-73.
- Kerner von Marilaun, A. 1891. "Pflanzenleben. Geschichte der Pflanzen," Vol. 2. Bibliographisches Institut, Leipzig.
- Kostoff, D. 1930. Tumors and other malformations on certain *Nicotiana* hybrids. *Zentr. Bakteriell Parasitenk., Abt. II.* **81**: 244-260.
- Kunkel, L. O. 1918. Tissue invasion by *Plasmodiophora brassicae*. *J. Agr. Research* **14**: 543-572.
- Kunkel, L. O. 1936. Heat treatments for the cure of yellows and other virus diseases of peach. *Phytopathology* **26**: 809-830.
- Kunkel, L. O. 1941. Heat cure of aster yellows in periwinkles. *Am. J. Botany* **28**: 761-769.
- Kunkel, L. O. 1944. General pathology of virus infections in plants. In "Handbuch



- der Virusforschung" (R. Doerr und C. Hallauer, eds.), 1. Ergänzungsband. Springer, Wien, pp. 473-521.
- Kunkel, L. O. 1951. Identification of bolting disease of carrots. *Phytopathology* **41**: 22. (Abstr.)
- Kunkel, L. O. 1954. Virus-induced abnormalities. *Brookhaven Symposia in Biol.* No. 6: 157-173.
- Kurosawa, E. 1926. Experimental studies on the secretion of *Fusarium heterosporum* on rice-plants. *J. Nat. Hist. Soc. Formosa* **16**: 213-227. (In Japanese; English abstr. in *Biol. Abstr.* **3**: 1066, 1929.)
- Küster, E. 1911. "Die Gallen der Pflanzen." Leipzig. 437 pp.
- Lacey, M. S. 1948. Studies on *Bacterium fascians*. V. Further observations on the pathological and physiological reactions of *Bact. fascians*. *Ann. Appl. Biol.* **35**: 572-581.
- Lang, A. 1956. Induction of flower formation in biennial *Hyoscyamus* by treatment with gibberellin. *Naturwissenschaften* **43**: 284-285.
- Lang, A. 1957. The effect of gibberellin upon flower formation. *Proc. Natl. Acad. Sci. U. S.* **43**: 709-717.
- La Rue, C. D. 1933a. Intumescences on poplar leaves. I. Structure and development. *Am. J. Botany* **20**: 1-17.
- La Rue, C. D. 1933b. Intumescences on poplar leaves. II. Physiological considerations. *Am. J. Botany* **20**: 159-175.
- La Rue, C. D. 1935. The rôle of auxin in the development of intumescences on poplar leaves; in the production of cell outgrowths in the tunnels of leaf-miners; and in the leaf-fall in *Coleus*. *Am. J. Botany* **22**: 908. (Abstr.)
- Lesley, J. W., and M. M. Lesley. 1928. The wiry tomato. *J. Heredity* **19**: 337-344.
- Lewis, I. F., and L. Walton. 1947. Initiation of the cone gall of witch hazel. *Science* **106**: 419-420.
- Lewis, I. F., and L. Walton. 1958. Gall-formation on *Hamamelis virginiana* resulting from material injected by the aphid *Hormaphis hamamelidis*. *Trans. Am. Microscop. Soc.* **77**: 146-200.
- Link, G. K. K. 1937. Role of heteroauxones in legume nodule formation, beneficial host effects of nodules, and soil fertility. *Nature* **140**: 507.
- Link, G. K. K., and V. Eggers. 1940. Avena coleoptile assay of ether extracts of nodules and roots of bean, soybean, and pea. *Botan. Gaz.* **101**: 650-657.
- Link, G. K. K., H. W. Wilcox, and A. D. Link. 1937. Responses of bean and tomato to *Phytomonas tumefaciens*, *P. tumefaciens* extracts,  $\beta$ -indoleacetic acid, and wounding. *Botan. Gaz.* **98**: 816-867.
- Littau, V. C., and L. M. Black. 1952. Spontaneous tumors in sweet clover. *Am. J. Botany* **39**: 191-194.
- Locke, S. B., A. J. Riker, and B. M. Duggar. 1938. Growth substance and the development of crown gall. *J. Agr. Research* **57**: 21-39.
- McCoy, E. 1929. A cytological and histological study of the root nodules of the bean, *Phaseolus vulgaris* L. *Zentr. Bakteriell. Parasitenk., Abt. II.* **79**: 394-412.
- McCoy, E. 1932. Infection of *Bact. radicola* in relation to the microchemistry of the host's cell walls. *Proc. Roy. Soc.* **B110**: 514-533.
- MacMillan, J., and P. J. Suter. 1958. The occurrence of gibberellin  $A_1$  in higher plants: isolation from the seed of runner bean (*Phaseolus multiflorus*). *Naturwissenschaften* **45**: 46.
- McMurtrey, J. E., Jr. 1932. Effect of thallium on growth of tobacco plants. *Science* **76**: 86.

- Maramorosch, K. 1957. Reversal of virus-caused stunting in plants by gibberellic acid. *Science* **126**: 651-652.
- Martin, J. P. 1942. Stem galls of sugar-cane induced with insect extracts. *Science* **96**: 39.
- Mendel, G. 1866. Versuche über Pflanzenhybriden. *Verhandl. naturforsch. Ver. Brünn.* **4**.
- Miller, C., and F. Skoog. 1953. Chemical control of bud formation in tobacco stem segments. *Am. J. Botany* **40**: 768-773.
- Molliard, M. 1912. Action hypertrophiante des produits élaborés par le *Rhizobium radicum* Beyer. *Compt. rend.* **155**: 1531-1534.
- Morel, G. 1948. Recherches sur la culture associée de parasites obligatoires et de tissus végétaux. *Ann. épiphyt.* [N.S.] **14**: 123-234 (*Sér. Pathol. vég. Mém.* 5).
- Moulton, J. E. 1942. Extraction of auxin from maize, from smut tumors of maize, and from *Ustilago zeae*. *Botan. Gaz.* **103**: 725-739.
- Mulholland, T. P. C., and G. Ward. 1954. Gibberellic acid. Part II. The structure and synthesis of gibberene. *J. Chem. Soc.* **1954**: 4676-4681.
- Näf, U. 1958. Studies on tumor formation in *Nicotiana* hybrids. I. The classification of the parents into two etiologically significant groups. *Growth* **22**: 167-180.
- Němec, B. 1910. "Das Problem der Befruchtungsvorgänge und andere zytologische Fragen," Chapter 6, Gebrüder Borntraeger, Berlin. pp. 151-173.
- Parr, T. 1940. *Asterolecanium variolosum*, Ratzeburg, a gall-forming coccid, and its effect upon the host trees. *Yale Univ. School Forestry Bull.* **46**. 49 pp.
- Pilet, P. E. 1952. Problème hormonal concernant l'*Endophyllum sempervivi* Lév. parasite du *Sempervivum tectorum*. *Ber. schweiz. botan. Ges.* **62**: 269-274.
- Pilet, P. E. 1953. Etude physiologique du parasitisme de l'*Uromyces Pisi* (Pers.) de By, sur l'*Euphorbia Cyparissias* L. *Experientia* **9**: 300-302.
- Plumb, G. H. 1953. The formation and development of the Norway spruce gall caused by *Adelges abietis* L. *Conn. Agr. Expt. Sta., Bull.* **566**: 1-77.
- Price, W. C., and C. Gainor. 1954. Relationship of foliage to stimulation of adventitious roots in crown gall. *Phytopathology* **44**: 107-109.
- Rasnizina, E. A. 1938. Formation of growth substances (auxin type) by bacteria. *Compt. rend. acad. sci. U. R. S. S.* **18**: 353-355.
- Reinert, J., and P. R. White. 1956. The cultivation *in vitro* of tumor tissues and normal tissues of *Picea glauca*. *Physiol. Plantarum* **9**: 177-189.
- Rössig, H. 1904. Von welchen Organen der Gallwespenlarven geht der Reiz zur Bildung der Pflanzengalle aus? *Zool. Jahrb. Abt. Allgem. Zool. Physiol. Tiere* **20**: 19-90.
- Ross, H., and H. Hedicke. 1927. "Die Pflanzengallen." Fischer, Jena.
- Schellenberg, H. C. 1915. Zur Kenntnis der Winterruhe in den Zweigen einiger Hexenbesen. *Ber. deut. Botan. Ges.* **33**: 118-126.
- Schweizer, J. 1933. Tjemaraziekte bij tabak. *Mededeel. Besoek. Proefsta.* **50**: 1-28.
- Skoog, F. 1944. Growth and organ formation in tobacco tissue cultures. *Am J. Botany* **31**: 19-24.
- Skoog, F. 1954. Substances involved in normal growth and differentiation of plants. *Brookhaven Symposia in Biol.* **No. 6**: 1-21.
- Skoog, F., and C. O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Symposia Soc. Exptl. Biol.*, **No. 11**: 118-131.
- Skoog, F., and C. Tsui. 1951. Growth substances and the formation of buds in plant tissues. In "Plant Growth Substances" (F. Skoog, ed.). Univ. Wisconsin Press, Madison, Wisconsin. pp. 263-285.

- Smith, E. F. 1914. "Bacteria in Relation to Plant Diseases," Vol. 3. Washington, D. C. 309 pp.
- Smith, E. F. 1920. "An Introduction to Bacterial Diseases of Plants." Saunders, Philadelphia, Pennsylvania. 688 pp.
- Smith, E. F., N. A. Brown, and L. McCulloch. 1912. The structure and development of crown gall: A plant cancer. *U. S. Dept. Agr., Bur. Plant Ind. Bull.* **255**: 61 pp.
- Sonneborn, T. M. 1946. Experimental control of the concentration of cytoplasmic genetic factors in *Paramecium*. *Cold Spring Harbor Symposia Quant. Biol.* **11**: 236-255.
- Sorauer, P. 1886. "Handbuch der Pflanzenkrankheiten," 2te neubearbeitete Auflage, Erster Teil. Blattauftrieb (Intumescencia). P. Parey, Berlin, pp. 222-227.
- Sparrow, A. H., J. E. Gunckel, L. A. Schairer, and G. L. Hagen. 1956. Tumor formation and other morphogenetic responses in an amphidiploid tobacco hybrid exposed to chronic gamma irradiation. *Am. J. Botany* **43**: 377-388.
- Spencer, E. L. 1935. Studies on frencing of tobacco. *Phytopathology* **25**: 1067-1084.
- Spencer, E. L., and G. I. Lavin. 1939. Frencing of tobacco. *Phytopathology* **29**: 502-503.
- Spiegelman, S. 1954. Heritable differences in enzyme synthesizing capacity amongst cells of identical genotype. *Proc. Natl. Cancer Conf. 2nd Conf.* 1954. **2**: 1345-1349.
- Steinberg, R. A. 1947. Growth responses of tobacco seedlings in aseptic culture to diffusates or some common soil bacteria. *J. Agr. Research* **75**: 199-206.
- Steinberg, R. A. 1950. The relation of certain soil bacteria to frencing symptoms of tobacco. *Bull. Torrey Botan. Club* **77**: 38-44.
- Steinberg, R. A. 1952. Frencing symptoms produced in *Nicotiana tabacum* and *Nicotiana rustica* with optical isomers of isoleucine and leucine and with *Bacillus cereus* toxin. *Plant Physiol.* **27**: 302-308.
- Steinberg, R. A., J. D. Bowling, and J. E. McMurtrey, Jr. 1950. Accumulation of free amino acids as a chemical basis for morphological symptoms in tobacco manifesting frencing and mineral deficiency symptoms. *Plant Physiol.* **25**: 279-288.
- Steward, F. C., and S. M. Caplin. 1951. A tissue culture from potato tuber: the synergistic action of 2,4-D and of coconut milk. *Science* **113**: 518-520.
- Stodola, F. H. 1956. Isolation, characterization, and chemical properties of the Gibberellins (Symp. Lecture.) *Am. Inst. Biol. Sci. Bull.* **6**, No. 4.
- Stodola, F. H., G. E. N. Nelson, and D. J. Spence. 1957. The separation of gibberellin A and gibberellic acid on buffered partition columns. *Arch. Biochem. Biophys.* **66**: 438-443.
- Stowe, B. B., and T. Yamaki. 1957. The history and physiological action of the gibberellins. *Ann. Rev. Plant Physiol.* **8**: 181-216.
- Takahashi, N., H. Kitamura, A. Kawarada, Y. Seta, M. Takai, S. Tamura, and Y. Sumiki. 1955. Biochemical studies on bakanae fungus. XXXIV. Isolation of gibberellins and their properties. *Bull. Agr. Chem. Soc. Japan* **19**: 267-277.
- Thimann, K. V. 1936. On the physiology of the formation of nodules on legume roots. *Proc. Natl. Acad. Sci. U. S.* **22**: 511-514.
- Thimann, K. V. 1939. The physiology of nodule formation. *Trans. Third Comm. Intern. Soc. Soil Sci.* **A**: 24-28.
- Thornton, H. G. 1936. The action of sodium nitrate upon the infection of lucerne root-hairs by nodule bacteria. *Proc. Roy. Soc.* **B119**: 474-492.

- Thornton, H. G. 1947. Report of the Department of Soil Microbiology for the Years 1939-1945. Rothamsted Expt. Sta. Harpenden, Herts, England.
- Thornton, H. G., and H. Nicol. 1936. Stimulation of root-hair growth in legumes by sterile secretions of nodule bacteria. *Nature* **137**: 494-495.
- Triggerson, C. J. 1914. A study of *Dryophanta erinacei* Mary and its gall. *Ann. Entomol. Soc. Am.* **7**: 1-46.
- Tschirch, A. 1890. Ueber durch *Astegopteryx*, eine neue Aphidengattung, erzeugte Zooecidien auf *Styrax Benzoin* Dryand. *Ber. deut. botan. Ges.* **8**: 48-53.
- Tubeuf, C. von. 1895. "Pflanzenkrankheiten, durch kryptogame Parasiten verursacht." Springer, Berlin, 600 pp.
- Wellman, F. L. 1941. Epinasty of tomato, one of the earliest symptoms of *Fusarium wilt*. *Phytopathology* **31**: 281-283.
- West, C. A., and B. O. Phinney. 1957. Purification and properties of gibberellin-like substances from flowering plants. *Plant Physiol. Suppl.* **32**: xxxii.
- White, O. E. 1948. Fasciation. *Botan. Rev.* **14**: 319-358.
- White, P. R. 1939. Controlled differentiation in a plant tissue culture. *Bull. Torrey Botan. Club.* **66**: 507-513.
- White, P. R. 1944. Transplantation of plant tumors of genetic origin. *Cancer Research* **4**: 791-794.
- White, P. R. 1945. Metastatic (graft) tumors of bacteria-free crown-galls on *Vinca rosea*. *Am. J. Botany* **32**: 237-241.
- White, P. R., and A. C. Braun. 1942. A cancerous neoplasm of plants. Autonomous bacteria-free crown-gall tissue. *Cancer Research* **2**: 597-617.
- White, P. R., and W. F. Millington. 1954a. The distribution and possible importance of a woody tumor on trees of the white spruce, *Picea glauca*. *Cancer Research* **14**: 128-134.
- White, P. R., and W. F. Millington. 1954b. The structure and development of a woody tumor affecting *Picea glauca*. *Am. J. Botany* **41**: 353-361.
- Wilson, P. W. 1940. "The Biochemistry of Symbiotic Nitrogen Fixation." Univ. Wisconsin Press, Madison, Wisconsin, 302 pp.
- Wipf, L., and D. C. Cooper. 1940. Somatic doubling of chromosomes and nodular infection in certain Leguminosae. *Am. J. Botany* **27**: 821-824.
- Wittwer, S. H., M. J. Bukovac, H. M. Sell, and L. E. Weller. 1957. Some effects of gibberellin on flowering and fruit setting. *Plant Physiol.* **32**: 39-41.
- Wolf, F. A. 1918. Intumescences, with a note on mechanical injury as a cause of their development. *J. Agr. Research* **13**: 253-260.
- Wolf, F. A. 1935. "Tobacco Diseases and Decays." Duke Univ. Press, Durham, North Carolina, 454 pp.
- Wolf, F. T. 1952. The production of indole acetic acid by *Ustilago zeae*, and its possible significance in tumor formation. *Proc. Natl. Acad. Sci. U. S.* **38**: 106-111.
- Yabuta, T., and T. Hayasi. 1939a. Biochemical studies on "bakanae" fungus of rice. II. Isolation of gibberellin, the active principle which produces slender rice seedlings. *J. Agr. Chem. Soc. Japan* **15**: 257-266. (*Chem. Abstr.* **33**: 8238, 1939.)
- Yabuta, T., and T. Hayasi. 1939b. Biochemical studies on "bakanae" fungus of the rice. III. Physiological action of gibberellin on the plants. *J. Agr. Chem. Soc. Japan* **15**: 403-413. (*Bull. Agr. Chem. Soc. Japan* **15**: 82-83, 1939, in English.)
- Yabuta, T., and T. Hayasi. 1940. Biochemical studies of "bakanae" fungus of rice. (In Japanese; English summary) *J. Imp. Agr. Expt. Sta. (Japan)* **3**: 365-400.



- Yabuta, T., and Y. J. Sumiki. 1938. [Isolation of gibberellin.] (In Japanese) *J. Agr. Chem. Soc. Japan* **14**: 1526. (Cited by B. E. Cross, *J. Chem. Soc.* 4670-4676, 1954.)
- Yabuta, F., Y. Sumiki, K. Aso, T. Tamura, H. Igarashi, and K. Tamari. 1941a. Biochemical studies of "bakanae" fungus. XI. The chemical constitution of gibberellin. 2. *J. Agr. Chem. Soc., Japan* **17**: 894-900. (*Chem. Abstr.* **44**: 10815, 1950.)
- Yabuta, T., Y. Sumiki, K. Aso, T. Tamura, H. Igarashi, and K. Tamari. 1941b. Biochemical studies of "bakanae" fungus. XII. The chemical constitution of gibberellin. 3. *J. Agr. Chem. Soc. Japan* **17**: 975-984. (*Chem. Abstr.* **44**: 10816, 1950.)

## CHAPTER 7

# Reproduction Is Affected

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## I. INTRODUCTION

Flowering plants must attain a minimum size or age before they can reproduce. During the vegetative stages of growth and development, a plant is especially dependent upon nutrients. Energy is required for the transition from vegetative to reproductive phases and for the process of

reproduction itself. Reserve foods supply this energy, and the plant, accordingly, must contain a sufficient reserve of foods to carry out the reproductive process. Reproductive organs, however, do not usually manufacture foods to any appreciable extent and seeds in particular are heterotrophic. In many species, especially those with an indeterminate growth habit, different parts of the same plant may simultaneously carry on vegetative and reproductive functions.

Light and temperature directly affect the initiation and development of floral primordia and the reproductive organs that develop later. Leaves, apical buds, and other vegetative organs are the receptors of these stimuli. Thus, the environment may determine whether anthesis begins, is retarded, or is suppressed. For example, sugar beets may flower prematurely in one environment or remain vegetative indefinitely in a humid environment. The course of reproduction may embrace more than a season, and, in biennials and woody plants, more than one year. Therefore, the plant is ordinarily subjected to wide variations in climate and nutrition during its life, factors that are critical to the over-all reproductive process.

Reproduction proceeds through stages of ripeness-to-flower, floral initiation, anthesis, fruit set, and maturation of fruit and seeds. As reproductive activity begins, the physiology of the plant is altered. Vegetative growth decreases and is continuously readjusted in accordance with the demands of reproduction. For a given plant and environment, the quantity of leaves, stems, and roots determines in a gross way the number of flowers formed and particularly the number of fruits that come to maturity. Especially in an unfavorable environment, there is a fluctuating competition between fruits and roots, leaves, or even other fruits on the same plant. As a consequence, reproduction slows down the vegetative growth but does not inhibit it entirely, and vegetative growth may proceed slower and then faster in succession in different stages of reproduction (Murneek, 1948). The complexity of these relationships is such that the plant as a whole must usually be considered in a discussion of the pathological aspects of reproduction.

Seeds and pericarp make very different substrates for pathogens. The heterogeneous nature of reproductive organs is evident on caryological examination. In addition, during the maturation of fruits, especially fleshy ones, seeds become dehydrated and show a marked increase in nitrogen content, and in condensation of reserve foods and finally appear isolated from the placenta, whereas in the pericarp, cell walls are hydrolyzed, simple sugars are formed, and water content increases.

Diseases of the pericarp have little influence on seeds unless there is premature fruit drop. Conversely, seeds may be killed by late frosts

whereas the pericarp is uninjured. When seeds are physiologically mature and firm, lysigenous breakdown of the pericarp begins adjacent to them and is intimately dependent upon their maturity (Naylor, 1952). On the other hand, the metabolic activities of the pericarp may produce physiologically active substances such as ethylene, which at appropriate concentrations may play a role in inhibiting seed germination. Thus, there is a correlation but no parallelism in the growth of the various parts of the fruit.

## II. PARTHENOCARPCIC AND UNDERDEVELOPED FRUITS

Seedless and parthenocarpic fruits exemplify the correlation in development of the fruit parts. When parthenocarpy occurs naturally, it is assumed that the auxin content of the unfertilized ovule is sufficient to promote fruit development. The same results can be obtained by the application of auxin sprays to plants that do not undergo parthenocarpy naturally. These fruits are seedless, contain sterile pseudo-seeds, or, by partial reversal of the normal growth correlation, parthenogenetic embryos, *sensu lato*.

In fruits, the embryo, albumen, and pericarp are correlated in development. The embryo and albumen both arise through separate fertilizations on the part of the pollen nuclei. Therefore, in sterile seeds with an

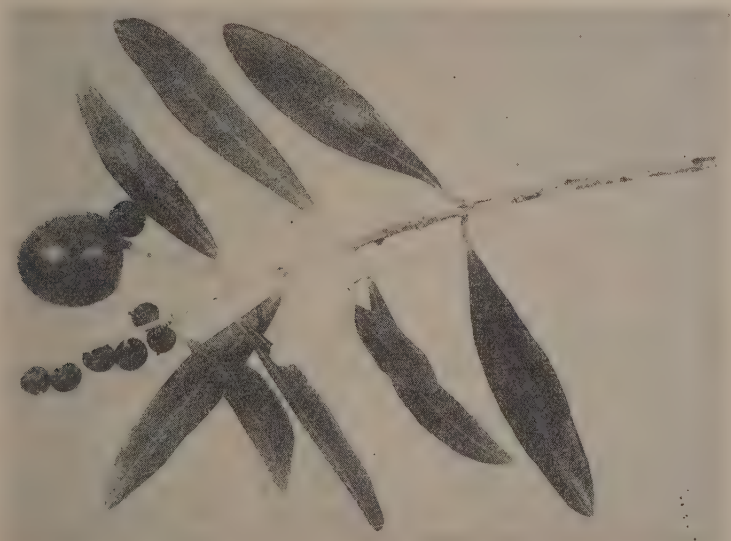


FIG. 1. Olive pseudodrupes (from Russo and Spina, 1952).



abnormal, nongerminative embryo, the endosperm may be quite normal, e.g., in *Ruta graveolens* (Cappelletti, 1929). A similar situation occurs in Bartlett pears (Griggs and Iwakiri, 1954). In a favorable environment, the Bartlett pear matures fruits that arise from vegetative or pollen-induced parthenocarpy and are produced uniformly, abundantly, and of good shape. Unfortunately in other cases parthenocarpy is partially induced.

In the olive tree, very small, persistent pseudodrupes originate (Fig. 1) from pollinated flowers in which elongation of the pollen tubes ceases in stylar tissues (Russo and Spina, 1952). In these fruits, the useless pericarp is complete in all its parts, whereas the stone which consists of the hardened endocarp, contains pseudoseeds showing parenchymatous degeneration of the 4 ovules and no embryo (Messeri, 1947). Factors that lead to arresting of pollen tube growth and therefore to the formation of pseudodrupes in olives are incompatibility factors (Russo and Spina, 1952), and unfavorable environmental conditions such as high humidity (Morettini, 1950) and low temperature (Petri, 1942).

Parasites may cause similar responses. Thus, olive pseudodrupes may be the consequence of attack by insects, such as *Coccus oleae* and *Mytilococcus ulmi* (Petri, 1927). The same intrinsic or environmental causes are thought to be responsible for some types of "shot-berries" in other fruits, e.g., grapes.

### III. NUTRITIVE DISTURBANCES

Nutritive diseases act on the whole plant. Consequently, they influence the reproductive organs through the vegetative ones. El Hinnawy (1956), who recently studied this problem, concluded that the influence of minerals on the flowering response of long and short day plants is indirect and is exerted through their effect on the production of auxin, and therefore on growth and the production of material for flower bud formation.

#### A. The Carbohydrate:Nitrogen Ratio

In past decades, there was a prevalent belief that the relation between the life of the individual and the preservation of the species was largely dependent upon the C:N ratio, the influence of which on flower initiation was expressed in Kleb's or Fischer's rule. All the conditions that either favor an accumulation of assimilates or hinder the absorption of water and nitrogen salts increase the ratio of carbohydrate to inorganic nitrogen and consequently favor the transition of the plant to the reproductive stage. On the other hand, all conditions which cause the opposite effects favor vegetative growth.

After the discovery of photoperiodism, the importance of C:N ratio

was questioned and flower induction is now thought to be controlled by flowering hormones—the florigens. These have not as yet been isolated.

Floral initiation is not fundamentally dependent upon the C and N supplies (Naylor, 1952). Rather the proper photoperiod and other environmental factors as well as nutrition are necessary conditions to stimulate the formation of florigens. In healthy plants, the transition from vegetative to reproductive stages requires a level of light and nutrition exceeding the minimum needed by vegetative growth (Lona, 1953). With the onset of reproduction, vegetative growth is reduced. N and particularly C accumulate in the plant, and as a result the C:N ratio increases. However, this accumulation seems a consequence of flower induction and not a cause of it.

Once floral primordia are initiated, they as well as the blossoms and fruits are strongly influenced by the C and N supplies and their broad interrelationships are very important. A satisfactory understanding of these complex phenomena has not been gained as yet; coordinate studies on growth promoting substances and on food competition throughout the plant are badly needed.

#### *B. Nutrient Excesses and Deficiencies Affecting Reproduction*

The above discussion of floral induction refers to plants living within “the limits of health”; if the abundance or the deficiency of one nutrient in relation to another exceeds a certain range, the plant enters into a real pathological condition.

The supply of carbon directly influences flowering through its presence in a carbohydrate precursor of florigens and through the influence of carbohydrate translocation on the movement of the flowering stimulus (Lincoln *et al.*, 1956).

Flower induction can be effected with very short exposures to illumination and with light intensities well below the compensation point. Later, a large supply of carbohydrates is necessary for reproductive growth and, at least in some cases, it has been found that carbohydrates directly influence the development of normal reproductive organs (Hartmann, 1955; Minessy and Schroeder, 1956).

The nitrogen nutrition of plants affects reproduction in a number of ways. Some plants seem to regulate nitrogen absorption regardless of the available supply (Crocker, 1948), but the response of different species to nitrogen is unpredictable. The assumption that nitrogen deficiency will promote flowering in long-day plants and slightly delay it in short-day plants proved to be erroneous (El Hinnawy, 1956); Murneek's conclusion (1948) still seems valid that nitrogen is one of the most crucial nutrient elements in the initiation of reproduction. Nitrogen

promotes growth in accordance with the principle that without nitrogen there is no growth hormone and consequently no growth (El Hinnawy, 1956). Therefore, an abundant nitrogen supply is usually required in the reproductive stages subsequent to floral induction. An excess or a deficiency of nitrogen can easily impair flowering or fruit set and the former condition frequently induces an increase in the number of members of flower verticillia, virescences, proliferations, and recrudescences of reproductive organs. Excessive nitrogen also increases the susceptibility of blossoms to low temperature (Boynton, 1954). During maturation of fruits, excessive nitrogen may excite more complex responses on the part of the plant as in "Baldwin spot" of apples (Garman and Mathis, 1956). Nitrogen deficiency may directly cause pistil abortion, affect pollen germination, and induce flower shedding and fruit drop. In dry climates, nitrogen deficiency is one of the most common causes of staminate flowers in the olive tree and nitrogen fertilization is, therefore, advocated so that otherwise symptomless trees will form normal flowers and set fruits (Petri, 1942). Biennial bearing in olives in dry climates is reduced by nitrogen dressings in April of the preceding year (Sommaini, 1954, 1955).

Although phosphorus does not affect flower initiation, according to El Hinnawy (1956), it accumulates in the developing primordia and flower buds. Phosphorus deficiency may lessen the fruit set and is a recognized factor affecting earliness of cereals and upland cotton, among other plants (Ergle and Eaton, 1957).

Phosphorus, however, does not seem highly important in some fruit crops. Thus, in peach, phosphorus can almost be considered a secondary nutrient, ranking below nitrogen, potassium, calcium, and magnesium (Bell and Childers, 1954). This behavior may be a result of the interaction of phosphorus with other elements, especially nitrogen.

Potassium plays an important role in carbohydrate metabolism; potassium deficiencies reduce set, size, sugar content, and color of fruits. An excess of available potassium, which is often related to high nitrogen levels, can indirectly damage reproduction because of its strong antagonism to calcium and, to a lesser extent, to magnesium, zinc, and other nutrients. Thus, an excess of potassium tends to accentuate the symptoms of blossom end-rot in tomatoes and of "Baldwin spot" in apples.

Boron deserves particular mention in regard to reproduction. In very low concentration (1 to 100 p.p.m.), it regulates the water absorption of certain pollens, prevents their bursting, and favors their germination. Also, it activates the development of the pollen tube. These functions of boron explain its presence in the nectar of the Nymphaeaceae and in the stigmatic secretions of tomato and other plants. The stimulation by boron of the elongation of pollen tubes is attributed to its influence on

oxygen uptake and sugar absorption, but it may also involve the synthesis of pectic materials in the cell walls of the elongating pollen tube (O'Kelley, 1957). In boron-deficient grapes, fruit set and yield are severely depressed, and fruits are seedless and undersized. Boron-deficient apples show internal and external corking. Preharvest drop and breakdown in late stored apples are also associated with the boron content.

A slight disturbance in boron nutrition sometimes affects a single stage of reproductive activity. Thus, an incipient boron deficiency in vigorously growing grapes and pears may cause failure of fruit set although there are no foliar symptoms. Sometimes this behavior is attributed to a high boron requirement of the plant when blossoming (Christ and Ulrich, 1954). In other cases it is attributed to the unavailability of boron in heavy, water-logged soils during the spring (Batjer *et al.*, 1953), or to the dilution of boron induced by rapid development of vegetation following nitrogen fertilization (Boynton, 1954).

According to Brennan and Shive (1948), the influence of calcium on carbohydrate translocation is a result of the relationship of calcium to boron. Recently, Joham (1957) has judged the influence of calcium on carbohydrate translocation to be similar to, but independent of, boron.

Calcium deficiency *per se* exerts other, more direct, influences on reproduction. Thus, in cotton it affects the earliness of flowering, the number of flowers, and the weight of bolls (Joham, 1957). "Baldwin spot" of apples, often attributed to an excessive development of foliage, has been associated with calcium deficiency in the fruit (Garman and Mathis, 1956).

A zinc deficiency prevents the normal production of tryptophan, a precursor of indoleacetic acid, and results in an increase in blossom and fruit drop.

Recently, iron-deficient cocklebur has been studied during photoinduction treatment. Staminate flower primordia were delayed in appearance and developed more slowly. In pistillate inflorescences, mature burs developed abnormally or failed to be produced. The influence of iron during photoinduction was more pronounced than that of boron or magnesium (Smith *et al.*, 1957).

Deficiency of magnesium seems especially effective in delaying flowering in such plants as mustard (El Hinnawy, 1956). It has also been repeatedly associated with fruit drop.

#### IV. WATER AND REPRODUCTION

When water in the soil or in the atmosphere is in excess or is deficient, or when the amount of water available undergoes sudden fluctuations, damage is likely to result. Unfavorable water relations in the soil



do not always produce the same effect as unfavorable moisture relations in the atmosphere. For instance, an excess of humidity around flowers may prevent the access of oxygen and reduce abscission, whereas an excess of water in the soil increases abscission.

Excessive moisture generally interferes with the transition of the plant to the reproductive stage. When the atmosphere is too humid, flowering is delayed, fertilization is reduced because insect movement is reduced, stigmatic secretions are diluted, and pollen grains are not dispersed by wind and burst. Consequently fruit set is curtailed.

High atmospheric humidity may affect reproduction, even in fully developed fruits and embryos. Wheat embryos may germinate in the inflorescences still in the field. Intumescences of pod valves and of bean and pea seed are also attributed to high humidity (Hiltner, 1933).

Drought conditions, even if short in duration, may have profound effects upon pollen formation and vitality. At meiosis, drought may cause diploid and tetraploid microspores and pollen grains in *Tradescantia*. If some wilting occurs, the pollen may fail to develop (Naylor, 1952). During bloom, water inadequacy, therefore, reduces fruit set and causes blossom shedding.

Later on, a moderate moisture deficiency may increase firmness and keeping quality of apples and pears, but—especially in arid climates—it affects the leaf: root and leaf: fruit ratios and the competition among fruits. Sometimes, when fruits have attained their final size, competition between fruits and vegetative organs favors the fruit itself. Thus, in the San Marzano variety of tomato, the leaves—which have a thin cuticle—may wilt, whereas the fruits hold their moisture more efficiently by means of the mucilages and pectic substances they contain, and mature almost normally.

Usually, however, a water deficit causes a reduction in size, number, and quality of mature fruits. In mild cases, there is a partially reversible withdrawal of water from fruits, causing a shrinkage in olives, citrus fruits, etc. (Savastano, 1934). For this reason, late spring and summer irrigation increases the frequency of annual bearing in the olive tree, lessens fruit drop, and increases the size of individual drupes (Hartmann, 1953). In temperate fruit crops, such as apple and pear, the flower buds are laid down in the year preceding flowering. A mild water stress during the critical period may slow down vegetative growth and enhance flower initiation (Tubbs, 1955). Once induction has occurred, the subsequent stages of flower development require an adequate water supply.

In some tropical fruit trees, the dry season is the only rest period and the succession of dry and rainy periods is necessary for a normal periodical growth. In *Litchi chinensis* floral initiation and flowering occur

satisfactorily in Canton (Southern China), where the above conditions are found, but floral initiation is rare in Hawaii and there recourse is made to the use of such auxins as sodium naphthalene acetate (Nakata, 1955).

Drought can prevent after-ripening and induce "secondary dormancy" in seeds.

## V. TEMPERATURE AND REPRODUCTION

In plants requiring a thermoperiod to go into the reproductive stage, temperature determines the initiation of flowering. The apical bud is the receptor of the stimulus and in the absence of a favorable thermoperiod, such plants either do not form floral primordia or fail to flower. If a favorable stimulus is received out of season, it can induce plants to flower at that time, e.g., the preflowering of sugar beets when winters are intermittently mild.

A minimum daily range of temperature is often necessary for good fruit set. Diurnal thermoperiodicity equilibrates photosynthesis and condensation reactions in daylight and respiration and growth at night. For the tomato, night temperatures should be at least 6° C. lower than daytime temperatures (Verkerk, 1955). The best temperatures for setting large crops of tomatoes in Texas, range from 13 to 20° C. at night and from 21 to 30° C. during the day (Young, 1957). Daily excursions of temperature are also needed for the after-ripening of some seed and can act as a substitute for light on light-sensitive seed.

Excursions of temperature on an annual basis are sometimes required. Thus, the annual periodicity of growth in subtropical trees is often impaired by the absence of a moderate winter chilling. Inflorescence development in olive and subsequent fruit production are generally proportional to the amount of chilling received (Hartmann and Porlingis, 1957). For this reason, olive yields poorly in the African highlands.

High temperatures are a common cause of blossom shedding and fruit drop. The most sensitive parts of flowers are pollen and stigmas. Thus, tomato pollen is inactivated when temperatures exceed 32° C. (Young, 1957) although the variety of the plant and its vigor affect this relation. Thus, the Hotset variety of tomato will set fruit at temperatures from 3 to 5° C. higher than the maxima that are usually tolerated.

Although high temperatures break dormancy of some seeds, they induce secondary dormancy in others and temperatures above 30° C. are not favorable to the germination of some species of seed (Toole *et al.*, 1955).

The first effect of low temperatures may be shown by pollen. Temperatures below 15° C. at the time of dispersal may devitalize maize

pollen (Elitropi, 1958). Reproductive organs are highly susceptible to frost injury. According to Modlibowska (1956), the various parts of the flower are not equally susceptible. Petals may be harmed when male and female organs are still uninjured and vice versa. Often the filaments are damaged, whereas the gynoecium is still intact. Early autumn frost may harm the flesh of fruits that are low in sugar at maturity or that ripen late in the fall or during the winter. Thus, according to Azzi (1928), mature olives are injured by temperatures of  $-0.4^{\circ}\text{C}$ . but even in these cases early frost causes little or no damage to reproduction.

Late spring frosts affect reproduction frequently. Modlibowska (1956) has shown that the economic consequences of late frost on the yield are less than is thought. Even when as many as 95% of the flowers are killed, the remaining ones produce a good crop if trees are well tended and pest and disease control is adequate. The effect of intense and repeated frosts on such early flowering trees as the almond may be so great, however, that the reproductive activities for the entire year may be suppressed.

The cold susceptibility of reproductive organs in different species varies widely. In apple and cherry the pistils are first injured at the base of the style. In pear the ovary is most susceptible. In cherry and plum the first lesions on the ovary are external. In apple and pear the centers of the flower and young fruit are injured earlier than the pericarp. Later the reverse is true. The susceptibility of flowers and fruits to frosts varies with the stage of growth, even in different varieties of a single species. Among apple varieties, "Belle de Boskoop" is equally sensitive at all stages; "Bramley's seedling" is most sensitive at the green button and pink button stages, but becomes hardier later; the reverse is true in "Ellison's orange" (Modlibowska, 1956).

The minimum temperature tolerated by flowers depends on their physiological condition, their nutrition, and the rapidity of growth. Thus rapid growth in a humid and warm environment predisposes flowers to frost injury.

Attack by pathogens may predispose flowers to frost injury. Thus, Modlibowska (1956) has shown that infection by *Stereum purpureum* increases susceptibility of plum flowers to low temperatures.

## VI. LIGHT AND REPRODUCTION

The requirement of plants for a certain duration of daylight (in order to flower) is now well known. In order to flower many plants require—in addition to a low temperature period—a short photoperiod of 10 hours or less, and others require one of 14 hours or more. The light stimulus is perceived by the leaves and the interval over which the response to day length occurs is that of photoperiodic induction.

For short-day plants, the dark period is the critical one if it has been preceded by a light period. Long-day plants flower even in continuous light and then failure to flower is due to an excessively long dark period. Some plants require a succession of two different photoperiods. Thus *Cestrum nocturnum* flowers when subjected to long days followed by short days or continuous long or short days or short days before long days are ineffective.

The behavior of short- and long-day plants is presently explained in terms of flowering hormones. Florigens, formed in leaves, would be destroyed by high levels of auxins. But when florigens are translocated to buds, auxins contribute to their fixation and to the subsequent differentiation of floral primordia (Salisbury, 1955). In floral induction, according to Liverman (1955), there is a lowering of the auxin level during the dark period in both long- and short-day plants. In short-day plants, the dark period causes the auxin content to drop to a level where florigen synthesis can occur. In long-day plants, the auxin level is too low for flowering under short-day conditions and long days are thus required for floral induction.

When occurring late in the life of a plant, photoperiodic stimuli sometimes cause abnormal responses. Thus short-day conditions may cause the formation of embryo sacs in the anthers and of pollen in the pistils (Naylor, 1952).

Even plants indifferent to day length, such as tomato, fail to fruit when exposed to day lengths of 5 hours or less or of 24 hours or more. In the latter case, foliar injury often occurs.

Photoinduction occurs both in woody and herbaceous plants. *Poinsettia* and *Bougainvillaea* are short-day woody plants and *Hibiscus syriacus* is a long-day plant. Other species are day neutral. Although in herbaceous plants, photoperiods induce flowering, in woody plants they control dormancy primarily (Wareing, 1956). Sometimes, as in apple flowering, climatic conditions are less important than physiological conditions and the latter are modified by climatic conditions in ways which are still obscure (Gorter, 1955). In tropical and subtropical plants, dormancy or vegetative pause seems to depend upon chilling or dry spells.

A number of growth regulators interfere with floral initiation, and Salisbury (1957) has identified the steps in photoperiod induction of cocklebur that are blocked by some growth regulators. One mechanism determines the critical day length. Other stages of induction are synthesis of flowering hormone in the leaf and development of floral buds. Evidence for this reasoning is based on the ability of cobaltous ion to interfere with the mechanism controlling critical day length; the inhibition by 2,4-dinitrophenol of florigen synthesis; the action of 3-indolacetic acid,



naphthalene acetic acid, and 2,4-D to destroy the flowering hormone in the leaf; and the ability of maleic hydrazide, Dalapon, and 2,4-D to inhibit floral bud development.

Quite apart from photoperiodic effects, intense light absorbed by dark surfaces such as fruits and flowers is converted into heat and the exposed tissues can attain temperatures 8 to 9° C. above the temperature of the surrounding air. Blueberries in New Jersey attained temperatures of 40° C. when the air was 31° C. (Stevens and Wilcox, 1918).

Intense light prevents the germination of many seeds, e.g., most Liliaceae, and induces secondary dormancy in some of them, e.g., *Nigella*, which become light hard. In such cases their germination is hindered, even when they are put again in darkness. Dark hardness may be caused by low light on light-requiring seeds, as, for example, many grasses.

In insufficient light, leaf production takes place at the expense of roots (Shirley, 1929) and reproduction is impaired. Delayed flowering, reduction in the initial number of flowers, shedding of blossoms, and slow development of fruits are usually induced by low light intensities, either at high or low temperatures.

When plants receive the full spectrum of daylight, intensity is not very important. About 40 foot-candles seem sufficient for mere survival of many plants. In Guthrie's experiments (1929), flowering was impaired only when illumination was reduced to 8% of full summer sunlight. When harmful effects occur, they usually result from an incomplete spectrum, which lacks the shorter green, blue, and violet wavelengths, i.e., shorter than 5200 Å. Under glass that fails to transmit violet light, some plants differentiate few if any flowers.

The studies of Flint and associates (in Crocker, 1948) were among the first to present modern information on the influence of different rays on the life of plants. According to them, the region 5200 to 7000 Å (red, orange, and yellow light) stimulates the germination of lettuce seeds, whereas the regions from 4200 to 5200 Å (green, blue, and violet) and 7000 to 8600 Å (mainly infrared) are inhibitive. The action spectrum for floral induction of short-day plants, such as *Xanthium pennsylvanicum*, is similar to that for germination of lettuce seed (Borthwick *et al.*, 1952). Present data suggest that red and infrared rays generally participate in flower induction and elicit other morphological responses of higher plants; so that the action of light upon some seeds would be one aspect of a general phenomenon influencing living processes (Was-sink and Stolwijk, 1956).

Red rays promote growth and flowering in barley, a long-day plant, whereas infrared rays inhibit growth and promote flowering in *Xanthium*,

a short-day plant. In flower initiation, the most effective red rays are in the region of 6500 Å and 7350 Å is the most effective region of the infrared. Red rays may repeatedly reverse the photoreactions induced by infrared and vice versa. These reactions appear to be independent of temperature. Their reversibility, however, is reduced by a dark period between the two irradiation periods. The effect of darkness depends upon temperature (Downs, 1957).

Infrared rays may also decrease fruit set, especially at high temperatures, in some neutral day plants, such as tomato (Young, 1957).

## VII. INTERACTIONS OF NUTRITION AND CLIMATE ON REPRODUCTION

Although the effects of nutrition and climate on reproduction have been examined separately, their action on plants is sometimes inextricably interrelated and cumulative. Thus, long- and short-day plants do not respond to photoperiodic stimuli when temperatures are too low (1 to 4° C.) or too high (30 to 38° C.) (Liverman, 1955). In short-day plants, the night temperature is very important, and in long-short day plants, e.g., *Cestrum nocturnum*, the day temperature has a great effect upon long-day induction, whereas the night temperature has its effect upon short-day induction (Sachs, 1956). During part of the photoperiodic cycle, cool temperatures are required by *Glycine max* (Blaney and Hamner, 1957), perhaps indicating an "endogenous rhythm" of 24 hours' duration (Bünning, 1956).

The behavior of seeds is altered by combinations of temperature and light. Lettuce seeds, kept for 24 hours at 25° C. in a dark germinator lose their sensitivity to light and do not respond to standard illumination. However, when stored in darkness at 5° C., they germinate normally. In a dry environment, seeds exposed to low temperatures do not after-ripen; but if allowed to imbibe water before exposure to red or infrared rays, the response of the seeds is different (Liverman, 1955).

The nature of the flowering response in cucurbits is altered by proper adjustment of temperature and light. Thus, cucurbits can be induced to form staminate, monoclinous, and pistillate flowers under appropriately controlled conditions, just as they do in nature (Nitsch *et al.*, 1952).

High temperature in association with low light intensity, induces poor fruit set in tomato as well as dormancy of fertilized ovaries (Johnson, 1956). In some fruits, high temperatures, intense light, and deficient moisture, in combination, alter cell permeability with the result that cell sap fills the intercellular spaces, parenchymal cells become discolored, and a watery decay of tissues sets in, e.g., water core of apples. Blossom end rot of tomatoes is another example. It can be induced by intense daylight, by high night temperatures (Verkerk, 1955), by temporary

water shortage in a critical stage of the development of the fruit, or by calcium deficiency in which the ratios of potassium to calcium and of soluble calcium to total salts in the soil solution are important (Geraldson, 1955).

"Shrunken grain" of wheat is another complex disease induced by moisture deficiency of the caryopses, which deficiency in turn is caused by hot dry winds, by pathogens such as rust or *Ophiobolus graminis*, or by malfunctioning of the vascular tissues, such as in lodging of wheat (Baldacci and Ciferri, 1944). According to Mulder (1951), bitter pit of apples is induced by inappropriate breeding and cultural methods, excess nitrogen, relative lack of phosphates due to magnesium deficiency, and adverse ratios of foliage to fruits.

#### VIII. IMPAIRED GROWTH CORRELATIONS OF REPRODUCTIVE ORGANS

The fulfilling of each stage of reproduction requires that the different organs be in the right condition at the right time. Thus, for fertilization to occur, pollen must be able to germinate, stigmas must be receptive; water, inorganic nutrients, sugars, and growth factors must all be adequate; and temperature and light must be within certain limits. If these conditions are not fulfilled, fertilization may be impaired. In tomato, high nitrogen levels, or low light, or high temperatures and high light intensity may induce style exsertion before dehiscence of the anther sacs occurs and, as a result, fruits fail to be set (Johnson, 1956; Leopold, 1955). In maize, the environmental conditions may easily induce the opposite condition, premature pollen dispersal, though this is usually not very harmful (Elitropi, 1958).

#### IX. PREMATURE ABSCISSION OF REPRODUCTIVE ORGANS

Histologically, when flowers and fruits drop prematurely, a dissolution of the middle lamellae and adjacent layers of the primary cell walls occurs in the abscission zone of the peduncle. Meristematic layers protect the stump of the peduncle, which remains on the plant, and the lumens of vessels become occluded by gums and tyloses.

Abscission is now thought to be controlled by the relative auxin concentration across the abscission zone. When the gradient of auxin across the abscission zone—from the proximal to the distal side—is steep, flowers and fruit are not shed; when the gradient is low, or disappears, or is reversed as a result of an auxin spray on leaves, abscission results (Addicott and Lynch, 1955). The "auxin gradient" hypothesis may not be generally valid, since in *Phaseolus vulgaris* and *Coleus blumei* the controlling factor in stimulation or inhibition of abscission is the total amount of auxin applied and not the auxin gradient (Gaur and Leopold, 1955).

In young flowers, auxin production is largely centered in the maturing stamens and ovaries (Leopold, 1955). When flowers are mature, their auxin content becomes very low, but upon pollination the ovary produces a new flush of auxin. This gradually diminishes until new auxin is produced by the endosperm and later by the embryo.

In the apple, there are four flushes of blossom and fruit abscission. The first drop is given by aborted and unfertilized flowers. The second appears associated with an insufficient activation of auxin-forming systems in the fertilized ovaries. These two can easily fuse one into another. The third (June drop) and the fourth (preharvest drop) occur in periods of lessened auxin production in endosperm and embryo. Auxin sprays satisfactorily control the first flushes of flower and fruit drop, when some environmental condition has temporarily interfered with pollination and limited fruit set.

Environmental conditions, however, can interfere with auxin action. Thus, Marglobe and Rutgers tomatoes do not respond to sprays of *p*-chlorophenoxyacetic acid under high temperatures and light intensities of summer, whereas summer setting varieties, sprayed with the same compound, nearly doubled their fruit production (Johnson and Hall, 1955). When there is a temporary lack of nutrients, as in the case of early tomatoes that may flower when there are still few leaves on the plant, auxin treatment can usefully delay fruit drop until nutrition becomes adequate, but auxins cannot indefinitely replace an adequate nutrient supply.

The competition for nutrients among reproductive organs may result in dropping of fruit shortly after fruit set. And, when the number of leaves is insufficient to supply the fertilized ovaries with the necessary food, the younger ovaries may become dormant because the fruit already set seems to have a priority upon the amount of nutrients and food available. In tomato and cotton, the dropping of young fruits is greater when older fruits have already been set on the plant. In cotton, for example, the dropping of fruits at the beginning of the fruit set period may be as low as 10%, whereas at the end of the flowering period, it may be over 90%. In this case, the abscission of younger fruits is stimulated by the auxin concentration of older fruits (Addicott and Lynch, 1955). Inasmuch as nutrients are translocated to and concentrated in regions high in auxin, young fruits receive fewer nutrients than older ones do.

The activity of auxins is, therefore, interrelated with and affected by nutrition as well as by light, temperature, respiration, and translocation (Hamner and Nanda, 1956; Biggs and Leopold, 1957). Furthermore, calcium is necessary for the formation of the insoluble pectins in middle lamellae and zinc is required for auxin synthesis. Carbohydrates are



specifically useful in the formation of cell wall material. Oxygen and carbon dioxide concentrations, insofar as they influence respiration at this stage, may influence abscission. The carbon:nitrogen ratio (a resultant of the processes of nutrition), photosynthesis, and respiration also provide information on the likelihood of abscission. Either a very high or a very low value of the carbon:nitrogen ratio indicates that fruit drop is likely.

Auxin sprays are a convenient method of preventing flower abscission, application being made to flowers or to the soil. A number of synthetic growth hormones are useful for this purpose. Esters of *p*-chlorophenoxyacetic acid, and of  $\beta$ -naphthoxyacetic acid are useful for improving fruit set of tomato. Very low concentrations of 2,4-D have proven effective on snap beans. Benzothiazole-2-oxyacetic acid, a weak auxin, is preferred for use on figs because it causes development of seed coats as well, and this has improved the saleability of the parthenocarpic fruit.

For the control of preharvest fruit drop, auxins are applied as sprays or aerosols and treatment is repeated. The treatment tends to improve coloring of apple fruits and does not increase the percentage of defective fruits. The use of auxins in this way has some unfortunate side effects. Thus, the respiratory rate of the fruit is increased so that the useful storage life is shortened. To counteract this effect, maleic hydrazide can be incorporated into the auxin spray. Auxins also enhance radial cracking in apples and induce puffiness and other undesirable effects in tomatoes. Unless the concentration of 2,4-D on oranges is at the low level of 10 mg. per liter it tends to induce abnormally large navels or seeded fruits in the otherwise seedless Navel orange.

Olives fail to respond to auxins sprays, applied to prevent preharvest drop. Melis (1949) has pointed out, however, that both preharvest and summer fruit drop of olive is often caused by infestations of *Prays oleellus*, the importance of which had been overlooked.

Other auxins have been used for fruit thinning. It is thought that they may act by inducing incompatibility between pollen tubes and stylar tissue or by inhibiting pollen germination or tube elongation (Leopold, 1955).

#### X. PHYSIOLOGICAL DISORDERS FAVORING THE ESTABLISHMENT OF PARASITES ON REPRODUCTIVE ORGANS

Physiological disturbances sometimes make the plant more susceptible to attack by parasites. Thus, intumescences of peas and other legumes, induced by high humidity, have been attributed to *Cladosporium pisi*, which only colonizes them. Grape inflorescences, injured by low temperatures, are usually invaded by *Botrytis cinerea*. Olive pistils are often

infected by *Pseudomonas savastanoi* through wounds caused by late frosts. Sunburn or sunscald of tomato is readily infected by ubiquitous molds such as *Alternaria tenuis*, *Cladosporium herbarum*, etc.

The colonization of necrotic tissues by common molds is so inevitable that some physiological disorders have long been mistaken for infectious diseases. Blossom end rot of tomatoes is an example. In southern Italy, the damage caused by *Monilia laxa* to almond blossoms is so dependent upon cold and humid weather that farmers attribute the rot and shedding of blossoms directly to unfavorable weather. In some of these diseases, the action of the pathogen is primary in importance although secondary in order of time. Such diseases might, therefore, be classed as "consequential" or "concatenate" diseases ("Folgekrankheiten" or "Kettenwirkung verschiedenartiger Krankheitsprocesse"; Morstatt, 1933).

## XI. INFECTIOUS DISEASES

Whether infectious diseases are systemic or local, they affect the general physiology of the host and, when the infection exceeds a certain intensity, reproductive activities of the host become affected. The same pathogen can behave differently in regard to host reproduction, depending on the mode and amount of invasion of host tissue. *Kunkelia nitens* prevents flowering of blackberry (*Rubus* spp.) when mycelium is generalized in host tissues from the rootstock to the growing point, but does not interfere with blossoming except in the invaded nodes when infection is local (Dodge, 1923).

Some pathogens of flowers, fruits, and seeds limit their attack to accessory parts of reproductive organs and thus do not interfere with the preservation of the species. *Coryneum beijerinckii* behaves in this way because it injures the bulky or dry pericarp of stone fruits.

### A. Seed-Borne Pathogens of the Vegetative Organs

Some parasites cause no injury to the seed but are simply carried on them and subsequently attack the plant developing from this seed. *Corynebacterium michiganense* plugs the micropyle and surrounds the embryo of tomato seeds, but may not interfere with the germination of the seed or with the life of the seedling. Thus, the disease is latent in the seed or seedling and becomes overt only in the vegetative organs of the adult plant. Similarly tomato plants, attacked by *Fusarium bulbigenum lycopersici*, often bear apparently normal fruits and viable seeds. In such cases, the reproductive organs act as carriers of a latent infection.

This situation is opposed to that of the parasite which generalizes in the green parts of the host plant and becomes pathogenic only in reproductive organs, e.g., many smuts.

### B. Diseases of the Vegetative Parts Specifically Affecting Reproduction

It is well known that parasites may stimulate distant responses, useful or injurious, on the part of their host plant. There are orchids (*Gastrodia elata*) that do not flower unless infected by *Armillaria mellea*, and *Rhizoctonia solani* induces the formation of aerial tubers on stems of potato plants infected in the roots and stolons.

Occasionally, responses unfavorable to reproduction are elicited by mycorrhizae, the importance of which in the normal physiology of many plants is well known. Mycorrhizae of the olive tree may intercept the major part of nitrogen available in a nitrogen deficient soil. Pistil abortion and blossom shedding ensue (Petri, 1914). Sometimes this nitrogen deficiency results in morphologically abnormal staminate flowers, as Petri (1942) and others have found.

In northern Italy, the nitrogen starvation resulting from mycorrhizae of *Ruta graveolens* has prevented the development of the embryo in otherwise normal seeds so generally that extinction of the species seems inevitable. Plants lacking mycorrhizae produce germinable seeds (Capelletti, 1929).

The indirect action of pathogens on reproduction is illustrated by *Gibberella fujikuroi*, which forms gibberellins in the plant. This fungus induces earliness in plants reaching adult size, but lowers yields. Generally, the gibberellins increase stem elongation and, in doing so, release a flowering response, especially evident in plants where the acceleration of vegetative growth eliminates mechanical barriers to flowering. The gibberellins may reduce germination time of seeds, but have no effect on total germination.

### C. Pathogens Attacking Reproductive Organs Directly

#### 1. Seed and Fruit Contaminants

Parasitic or saprophytic microorganisms may get into the flower and be borne on the surface of the seeds as external contaminants; or they may penetrate into its external layers, inducing "transitional infections," "pseudoflower infections," or "flower-seedling infections" (Gäumann, 1950). These microorganisms interfere with the viability of the embryo only when favored by particular environmental conditions. Among the genera of fungi acting in this manner are *Alternaria*, *Cladosporium*, *Helminthosporium*, *Pullularia*, and *Stemphylium*. "Heating" of moist grains is due to these fungi.

Most of the parasitic fungi that act in this way infect the seedling during preemergence or immediately thereafter. Some, e.g., *Helminthosporium gramineum*, are also harmful later in the life of the plant.

## 2. Fructicolous Infections

Some pathogens attack reproductive organs only. Thus the flower primordia of *Pennisetum* are attacked by *Tolyposporium penicillariae*, monoclinous flowers ready to blossom are attacked by *Claviceps purpurea*, and entire female flowers of *Ulmus* are attacked by *Taphrina* spp. Pistils are also attacked. Sometimes they are partially infected, as in "partial bunt" of wheat caused by *Tilletia indica*. In others they are entirely invaded, as in the case of mulberry ovaries attacked by *Ciboria carunculoides*. The seeds of *Alnus* are attacked by *Helotium seminicola* and fruits may also be attacked as in the case of *Tilletia barclayana* of rice.

These parasites sometimes enter sexual organs through the accessory parts of the flower or fruit. In other cases they infect the pistils directly or are inoculated by insects into seeds or fruits, e.g., stigmatomycoses.

Many pathogens overwinter in the affected organs of the host, some in the soil by their own perennating structures, as in the case of *Claviceps purpurea* and many smuts; still others survive in ways that are not yet known (stigmatomycoses). These pathogens are not externally seed-borne, do not induce generalized infections of their hosts, and might be considered "fructicolous" *sensu lato*.

## 3. Generalized Parasites with Sporulation Restricted to Reproductive Organs

Other pathogens, perhaps the most interesting for students of diseases of reproduction, are more or less generalized through the green organs, where their parasitism is more or less indifferent to the host plant. They sporulate and, in doing so, induce overt disease in reproductive organs or in the accessory organs of flowers and fruits. Gäumann (1950) considers these as agents of "organotropic diseases."

In rare cases the infection begins from the embryo, which becomes infected at anthesis. *Ustilago nuda* and *U. tritici* provide examples of this condition. Gäumann (1950) has used the term "germinative transmission" to describe this situation and Fischer and Holton (1957) used the term "embryo infection." In other cases, infection may begin in the seed coat, e.g., *Botrytis anthophila*; in other instances, it arises from the pericarp and husks, e.g., *Ustilago avenae*, from the seedlings or from the shoots, as in *Ustilago violacea*. The period of incubation of shoot infections may last more than one year in plurennials. Parasites causing seedling infections and those causing seed coat, pericarp, and husk infections have seed-borne or soil-borne spores or mycelia. These situations correspond to Gäumann's (1950) terms "pseudoflower infection," "flower-seedling infection," and "adherent transmission." Such modes of infec-



tion may coexist in the same species. Thus, *U. violacea* can penetrate into the plant through flowers, buds, and excised stems (Spencer and White, 1951).

The chrysanthemum aspermy virus causes deformation of flowers but no symptoms or at most a slight mottling on leaves. The virus can be recovered from roots, stems, leaves, and flowers; sap from flowers is the most infective, that from roots the least. Leaf sap is said to contain an inhibitor, although little or none is present in the flowers (Hollings, 1955). Therefore, this is a case of "forced organotropism" (Gäumann, 1950).

The diseases discussed in Sections XI, C, 2 and 3 have been termed "venereal diseases" by Gäumann (1950). This is a suggestive term. Venereal diseases, however ("venereal" from Venus, the goddess of love) are not such simply because they affect the reproductive organs, but because they usually arise from sexual intercourse with an infected mate. Thus cancer of the reproductive organs is not a venereal disease. In the diseases considered above, the pathogens infect the reproductive organs but this is independent of the fertilization processes which are often prevented by the diseases caused by the pathogens.

In some cases, they effect also a "parasitic castration" (*Ustilago violacea*) or induce a "parasitic unfruitfulness" in a wider sense. Commonly, they do not penetrate into the host through the stigma and the stylar tissue. Even *U. tritici*, which shows so high an electivity for embryos, pierces the wall of the young ovary laterally (Batts, 1955; Batts and Jeater, 1958; Ohms, 1955).

It seems interesting to note that an apomictic progeny of a male sterile mutant of *Paspalum notatum* produced more than five times as many ergotized florets, infected by *Claviceps paspali*, as a similar progeny of a male fertile sister plant tested in comparison (Burton and Lefebvre, 1948). The sphacelial stage of *Claviceps purpurea* developed even on florets of wheat deprived of stamens and ovaries (Cherewick, 1953); *Tilletia buchloeana* and *Sorosporium Everhartii* may transform into bunt or smut balls the rudimentary ovaries of staminate spikelets, which normally would never produce seeds (Fischer and Holton, 1957). Therefore, these fungi are to a certain degree independent from the presence of mature sexual organs and from the nutritive environment including hormones and similar substances associated with the reproductive state.

A similarity to venereal diseases may be shown only by those diseases which are transmissible by way of the fertilization process. This is the case of bean mosaic virus (*Phaseolus virus 1*), which can be trans-

mitted through the agency of the pollen of infected plants. This virus disease, however, is also transmitted in other ways and, when transmitted through the pollen, does not particularly damage reproduction.

#### 4. *Reproduction Affected by Nonelective Parasites Entering the Plant through Reproductive Parts*

Still other parasites (*Erwinia amylovora* and *Sclerotinia* spp., especially *S. laxa*) are capable of attacking tender vegetative organs, such as unfolding buds and young leaves or twigs through natural openings and through wounds, but they frequently penetrate the plant through floral organs such as petals, stamens, and pistils. These structures are either not cuticularized or little so, and offer conditions conducive to infection: high humidity and the presence of nectar and stigmatic secretions. Ubiquitous organisms may also infect stigmas and enter fruits where they usually remain ineffective, as, for example *Bacillus vulgatus* in *Cucurbita pepo* (Marcus, 1942). High atmospheric humidity favors the infection process. Thus, according to Weaver (1950), at 90% relative humidity peach blossoms contract infection by *Sclerotinia fructicola* through all floral organs; at 70 to 80% relative humidity, blight occurs only from infected stigmas; while at 70% relative humidity, blight of blossoms occurs only from stamen infections.

In the stone fruits, flower buds open before foliar buds do, and when the season is most favorable to infection, floral infections are abundant. In other cases, as with *Erwinia amylovora*, flowers attract the insect vectors of the pathogens rather than the pathogens themselves.

Some pathogens may cause late infections on maturing fruits, entering through lenticels or by piercing the cuticle as, for example, *Sclerotinia* spp. In these fruits, which are changed into mummies, the perfect stage of the pathogen is differentiated. Thus, fruits infected late act as primary host organs and flowers and vegetative parts as secondary host organs.

#### D. *Undesirability of Sharp Discrimination between Vegetative and Reproductive Organs as Substrates for Pathogens*

Gäumann (1950) rightly points out that sexual organs are the best individualized organs of the plant body. This individuality refers not only to the morphology and to the tissue characters of the reproductive organs, but also to the microenvironment they offer, which is peculiar and favorable to most pathogens, elective or not. Furthermore, in annual plants and in cereals in particular, seeds are the organs which permit the nonperennating structures of the parasites to survive best. Several exam-

ples have already been given to show that the suitability of reproductive organs for pathogens is not uniform through the flower and fruit.

Many pathogens affecting flowers, fruits, or seeds are able to sporulate on or to infect certain reproductive organs only, such as stamens and pistils. Among these pathogens are some of the least specialized which are provided with the grossest symbiotic adjustments. Electivity may be so strict that in some instances infection or sporulation can occur only on stamens, as, for example, *Ustilago violacea* and *Botrytis anthophila*, or on stigmas and filaments, e.g., *Peronospora stigmaticola*, or on peduncles, e.g., *Mycosyrinx cissi*. Others are restricted to petals, e.g., *Peronospora corollae*, seed funicles and placentae, as is the case for *Ustilago duriaeanae* and *Schroeteria* spp. Viennot-Bourgin (1953) distinguished two types of floral galls induced by the Ustilaginales. In the first type, chlamydospores arise only in the stamens, the external flower verticillia not showing evident anomalies. In the second type, the whole of the floral organ, except stamens, participate in chlamydospore production.

Most parasites which overwinter as resting mycelium in viable seeds or fruits do not invade or injure the embryo, and *Ustilago tritici*, which invades the embryo, is elective for the scutellum and the growing point region and does not penetrate into the radicle (Batts and Jeater, 1958). As stated above, even parasites showing a less high degree of parasitism may not behave indiscriminately toward the different parts of the flowers and fruits. *Ustilaginoides virens* does not affect stamens and pollen grains (Hashioka *et al.*, 1951), and the filaments of the flowers of sour cherry are infected by *Sclerotinia laxa* (which displays poor electivity) only through wounds (Calavan and Keitt, 1948).

All the organs of the flower are affected by *Claviceps purpurea*, which parasitizes even the remnants of the glumes in heads invaded by *Ustilago tritici*, but *C. purpurea* tends to form sclerotia, and consequently ergotamine and related compounds, only in the presence of the ovary (Cherewick, 1953). This is detached from the receptacle and remains at the apex of the developing sclerotium (Ramstad and Gjerstad, 1955).

The foregoing examples suggest that many parasites are elective for single organs, and the activity of these pathogens is independent of the reproductive activity of the host. In some cases, however, infection is coordinated with the fertilization process. *Claviceps purpurea* commonly infects wheat before, and *Ustilago tritici* after the fertilization of the ovule.

That the activity of the pathogen is, in fact, independent of reproductive activity may be shown by experimentation or through field observation by demonstrating that green tissues, which are normally

not invaded or injured by the elective parasites considered here, are at least as subject to attack by these fungi as the reproductive organs. Some conditions favor such fungi as *Tilletia caries*, *T. foetida*, and *Ustilago tritici* to sporulate on vegetative organs (see Grasso, 1953 for literature), although these fungi usually manifest themselves in the inflorescence of wheat. The greenhouse environment, the amputation of the inflorescence (Flor, 1932), and the infection of lemmas by *Trichothecium roseum* or *Fusarium culmorum* (Viennot-Bourgin, 1953)—all favor this type of action. Viennot-Bourgin (1937), therefore, thinks that the parasitic action of *Ustilago nuda* f. sp. *tritici* makes it possible to approximate the Ustilaginales localized in vegetative organs to the naked smuts, usually considered specific for the inflorescences; and that a transition exists between the smuts sporulating on the ovaries, on the flower involucre, and on leaves (*Ustilago maydis*) (Viennot-Bourgin, 1953).

The best known fructicolous fungi show similar aspects and the conclusion of Cherewick (1953) about *Claviceps purpurea* is that ergot may develop not only on young ovaries but on any physiologically young tissue of the wheat and barley plant.

Sometimes the behavior of the pathogen is more complex. It changes, although the host organs and environmental conditions are equivalent. Maybe in these cases, the stage of the disease reveals its importance. Why do some Ustilaginales, e.g., *Tilletia barclayana* and *T. indica*, soon sporulate in ovaries on which their wind-borne conidia alight, whereas others, e.g., *Ustilago tritici*, sporulate only in the flowers they enter from adjacent vegetative tissues that are generally invaded? What changes does the host-parasite complex go through during the development of the infected wheat plant?

Many parasites affecting reproduction enter the plant through its vegetative organs and can be controlled by protective fungicides or by the natural defenses of the host during the process of infection. We might, therefore, conclude that the behavior of the green parts of the plant is of even more interest than that of the reproductive parts on which the pathogens usually manifest themselves.

## XII. THE INFLUENCE OF REPRODUCTION ON THE ACTIVITY OF PATHOGENS AND DISEASE EXPRESSION

The stimuli for the transition of the plant from the vegetative to the flowering condition excite responses throughout the plant, and influence the plant in its entirety. Therefore, even in diseases not especially related to reproduction and to the organs directly responsible for it, maturity and reproductive activity often upset the equilibrium between host and pathogen.



In some cases, the flowering plant overcomes its invaders, as in the case of root nodule bacteria on legumes. More often, the reverse is true. The attack of mature plants by rusts, the epidemic phase of *Phytophthora infestans* (Grainger, 1957), the leaf target spot stage of *Alternaria solani*, the *Oidia* as agents of "Alterskrankheiten," all afford examples of micro-organisms more or less favored in this way. Genetic, seasonal, ecological, and nutritive factors, separately or in association, can also play a role in these changes of the physicochemical environment of host tissues. Recently, Grainger (1957) has proposed an index for the interpretation of the differences in susceptibility of new sprouts, young plants, and mature potato plants to *Phytophthora infestans*. This index indicates susceptibility or resistance according to its high or low value and consists of the ratio of the weight of total carbohydrates in the whole plant,  $C_p$ , divided by the residual dry weight of the shoot,  $R_s$ . The ratio  $C_p : R_s$  has been usefully adapted by Grainger for other diseases as well. The study of tissue factors, which are directly responsible for arresting the progress of infection or for allowing it to evolve into overt disease, is still in a most primitive state in animal and plant pathology (Dubos, 1954).

It is sufficient to conclude here that young vegetation may resist infection or keep it latent and be a "carrier" of disease, which becomes overt when the plant is mature. In such cases, the behavior of the young plant can be opposed to that of the same plant when adult. In these cases, therefore, the determinants of the pathological process are not the infectious agents, but the physiological changes of the host plant at maturity.

From a very different point of view, reproduction is a useful tool in checking the spread of and the economic effects of infectious diseases. Vegetative propagation results in genetic uniformity. Fertilization, and especially cross-fertilization, by contrast, makes for genetic heterogeneity. Stevens (1948), analyzing disease damages in clonal and pollinated crops, found that disease was more frequent on the former than on the latter and that losses were least in cross-fertilized plants.

### XIII. REPRODUCTIVE ORGANS AS INJURIOUS AGENTS

Occasionally, pollen grains are harmful to human beings and injure vegetation, too. Valteau (1949) demonstrated that the leaf bleaching of tobacco growing near corn is caused by corn pollen on the tobacco plants. The reasons for this response are unknown.

### REFERENCES

- Addicott, F. T., and R. S. Lynch. 1955. Physiology of abscission. *Ann. Rev. Plant Physiol.* 6: 211-238.  
Azzi, G. 1928. "Ecologia agraria." U.T.E.T., Torino. 237 pp.

- Baldacci, E., and R. Ciferri. 1944. Studi sulla "stretta dei cereali. Prima contribuzione. *Atti ist. botan. Univ. Lab. Crittogam. Pavia Atti Ser. 5* 1: 217-276.
- Batjer, L. P., B. L. Rogers, and A. H. Thompson. 1953. Blossom blast of Pears; an incipient Boron deficiency. *Proc. Am. Soc. Hort. Sci.* 62: 119-122.
- Batts, C. C. V. 1955. Infection of wheat by loose smut, *Ustilago tritici* (Pers.) Rostr. *Nature* 175: 467-468.
- Batts, C. C. V., and A. Jeater. 1958. The development of loose smut (*Ustilago tritici*) in susceptible varieties of wheat, and some observations on field infection. *Brit. Mycol. Soc. Trans.* 41: 115-125.
- Bell, H. K., and N. F. Childers. 1954. Peach Nutrition. In "Fruit Nutrition" (N. F. Childers, ed.), Chapter 10. Somerset, Somerville, New Jersey, pp. 495-641.
- Biggs, R. H., and A. C. Leopold. 1957. Factors influencing abscission. *Plant Physiol.* 32: 626-632.
- Blaney, L. T., and K. C. Hamner. 1957. Interrelations among effects of temperature, photoperiod, and dark period on floral initiation of Biloxi soybean. *Botan. Gaz.* 119: 10-24.
- Borthwick, H. A., S. B. Hendricks, M. W. Parker, E. H. Toole, and V. K. Toole. 1952. A reversible photoreaction controlling seed germination. *Proc. Natl. Acad. Sci. U. S.* 38: 662-666.
- Boynton, D. 1954. Apple Nutrition. In "Fruit Nutrition" (N. F. Childers, ed.), Chapter 1. Somerset, Somerville, New Jersey, pp. 1-78.
- Brennan, E. G., and J. W. Shive. 1948. Effect of calcium and boron nutrition of the tomato on the relation between these elements in the tissue. *Soil Sci.* 66: 65-75.
- Bünning, E. 1956. Endogenous rhythms in plants. *Ann. Rev. Plant Physiol.* 7: 71-90.
- Burton, G. H., and C. L. Lefebvre. 1948. Ergot and sterility in Bahia grass. *Phytopathology* 38: 556-559.
- Calavan, E. C., and G. W. Keitt. 1948. Blossom and spur blight (*Sclerotinia laxa*) of sour cherry. *Phytopathology* 38: 857-882.
- Cappelletti, C. 1929. Sterilità di origine micotica nella "*Ruta patavina*" L. *Annali botan.* 18: 145-166.
- Cherewick, W. J. 1953. Association of ergot with loose smut of wheat and of barley. *Phytopathology* 43: 461-463.
- Christ, E. G., and A. Ulrich. 1954. Grape nutrition. In "Fruit Nutrition" (N. F. Childers, ed.), Chapter 8. Somerset, Somerville, New Jersey, pp. 295-343.
- Crocker, W. 1948. "Growth of Plants. Twenty Years' Research at Boyce Thompson Institute." Reinhold, New York. 459 pp.
- Dodge, B. O. 1923. Systemic infections of Rubus with the orange rusts. *J. Agr. Research* 25: 209-242.
- Downs, R. J. 1957. Photoreversibility of flower initiation. *Plant Physiol.* 31: 279-284.
- Dubos, R. J. 1954. "Biochemical Determinants of Microbial Diseases." Harvard Univ. Press, Cambridge, Massachusetts. 152 pp.
- El Hinawy, E. I. 1956. Some aspects of mineral nutrition and flowering. *Mededeel. Landbouwhogeschool Wageningen* 56: 1-51.
- Elitropi, C. 1958. Contributo alla conoscenza della biologia florale e della tecnica d'impollinazione artificiale del Mais (*Zea Mays* L.). Parte II and Parte III. *Ann. sper. agrar. (Rome)* [N.S.] 12: 5-33, 35-54.
- Ergle, D. R., and F. M. Eaton. 1957. Aspects of phosphorus metabolism in the cotton plant. *Plant Physiol.* 32: 106-113.
- Fischer, G. W., and C. S. Holton. 1957. "Biology and Control of the Smut Fungi." Ronald, New York. 622 pp.

- Flor, H. H. 1932. The production of bunt chlamydospores in the vegetative tissue of the wheat plant. *Phytopathology* **22**: 661-664.
- Gäumann, P., and W. T. Mathus. 1956. Studies of mineral balance as related to occurrence of Baldwin Spot in Connecticut. *Conn. Agr. Expt. Sta. (New Haven) Bull.* **601**: 19 pp.
- Gäumann, E. 1950. "Principles of Plant Infection" (transl. by W. B. Brierley). Crosby Lockwood & Son, London. 543 pp.
- Gaur, B. K., and A. C. Leopold. 1955. The promotion of abscission by auxin. *Plant Physiol.* **30**: 487-490.
- Geraldson, C. M. 1955. The use of calcium for control of blossom-end rot of tomatoes. *Proc. Florida State Hort. Soc.* **68**: 197-202.
- Gorter, C. J. 1955. Photoperiodism of flowering in apple trees. *Rept. Intern. Hort. Congr. 14th Congr., London* **1**: 351-354.
- Grainger, J. 1957. Blight—the potato versus *Phytophthora infestans*. *Agr. Rev. (London)* **3**: 10-26.
- Grasso, V. 1955. Attacchi di *Ustilago hordei* (Pers.) Lagerh. sui culmi dell'Orzo. *Ann. sper. agrar. (Rome)* [N.S.] **7**: 1087-1093.
- Griggs, W. H., and B. T. Iwakiri. 1954. Pollination and parthenocarp in the production of Bartlett pears in California. *Hilgardia* **22**: 643-678.
- Guthrie, J. D. 1929. Effect of environmental conditions on the chloroplast pigments. *Am. J. Botany* **16**: 716-746.
- Hamner, K. C., and K. K. Nanda. 1956. A relationship between applications of indoleacetic acid and the high-intensity-light reactions of photoperiodism. *Botan. Gaz.* **118**: 13-18.
- Hartmann, H. T. 1953. Olive production in California. *Calif. Agr. Expt. Sta. Ext. Serv. Manual* **7**: 59 pp.
- Hartmann, H. T. 1955. Induction of abscission of olive fruits by maleic hydrazide. *Botan. Gaz.* **117**: 24-28.
- Hartmann, H. T., and I. Porlingis. 1957. Effect of different amounts of winter chilling on fruitfulness of several olive varieties. *Botan. Gaz.* **119**: 102-104.
- Hashikata, Y., M. Yoshino, and T. Yamamoto. 1951. Physiology of the rice false smut, *Ustilagoidea virens* (Cke.) Tak. *Research Bull. Saitama Expt. Sta.* **2**: 20 pp.
- Hiltner, E. 1933. Kälte und Witterung als Ursachen nichtparasitärer Pflanzenkrankheiten. In P. Sorauer's "Handbuch der Pflanzenkrankheiten," Band 1, 1 Teil, "Die nichtparasitären und Virus-Krankheiten" (O. Appel, ed.), Sechste Aufl., Spez. Teil, II Abs. Parey, Berlin. pp. 318-474.
- Hollings, M. 1955. Investigations of chrysanthemum viruses. I. Aspermy flower distortion. *Ann. Appl. Biol.* **43**: 86-102.
- Joham, H. E. 1957. Carbohydrate distribution as affected by calcium deficiency in cotton. *Plant Physiol.* **32**: 113-117.
- Johnson, S. P. 1956. Influence of growth regulators on setting of tomato fruits: a concept. *Proc. Am. Soc. Hort. Sci.* **67**: 365-368.
- Johnson, S. P., and W. C. Hall. 1955. Further studies on vegetative and fruiting responses of tomatoes to high temperature and light intensity. *Botan. Gaz.* **117**: 100-113.
- Leopold, A. C. 1955. "Auxins and Plant Growth." Univ. Calif. Press, Berkeley. 354 pp.
- Lincoln, R. G., K. A. Raven, and K. C. Hamner. 1956. Certain factors influencing expression of the flowering stimulus in *Xanthium*. Part I. Translocation and inhibition of the flowering stimulus. *Botan. Gaz.* **117**: 193-206.
- Liverman, J. L. 1955. The physiology of flowering. *Ann. Rev. Plant Physiol.* **6**: 177-210.

- Lona, F. 1953. La reazione fotoperiodica con speciale riguardo ai processi biochimici della fotofase. I. — I processi euflogeni. *Nuovo giorn. botan. ital.* [N.S.] **40**: 851-857.
- Marcus, O. 1942. Ueber das Vorkommen von Mikroorganismen in pflanzlichen Geweben (nach Untersuchungen an Früchten und Samen). *Arch. Mikrobiol.* **13**: 1-44.
- Melis, A. 1949. Nuovo contributo alla ricerca dei mezzi di lotta per combattere gli individui della generazione carpofaga della Tignola dell'Olio (*Prays oleellus* F.). *Redia* **34**: 83-123.
- Messeri, A. 1947. Osservazioni morfologiche sulle "olive passerine." *Nuovo giorn. botan. ital.* [N.S.] **54**: 374-376.
- Minessy, F. A., and C. A. Schroeder. 1956. Pistil development in citrus flowers. *Botan. Gaz.* **117**: 343-347.
- Modlibowska, I. 1956. Le problème des gelées printanières et la culture fruitière. *Congr. Pomol. Intern. Namur, Rapport Général* pp. 83-112.
- Morettini, A. 1950. "Olivicoltura" Ramo Ed. degli Agric., Roma. 595 pp.
- Morstatt, H. 1933. Allgemeine Pflanzenpathologie. In P. Sorauer's "Handbuch der Pflanzenkrankheiten," Band 1, I Teil, "Die nichtparasitären und Virus-Krankheiten" (O. Appel, ed.), Sechste Aufl., Allgem. Teil, II Abs. Parey, Berlin. pp. 80-198.
- Mulder, D. 1951. Stip in Apples als cultuurverschynsel. *Mededeel. Directeur Tuinb., Wageningen* **14**: 26-67.
- Murneek, A. E. 1948. Nutrition and metabolism as related to photoperiodism. In "Vernalization and Photoperiodism" (A. E. Murneek and R. O. Whyte, eds.). *Chronica Botanica*, Waltham, Massachusetts. pp. 83-90.
- Nakata, S. 1955. Floral initiation and fruit-set in lychee, with special reference to the effect of sodium naphthaleneacetate. *Botan. Gaz.* **117**: 126-134.
- Naylor, A. W. 1952. Physiology of reproduction in plants. *Survey Biol. Progr.* **2**: 259-300.
- Nitsch, J. P., E. B. Kurtz, J. L. Liverman, and F. W. Went. 1952. The development of sex expression in cucurbit flowers. *Am. J. Botan.* **39**: 32-43.
- Ohms, R. E. 1955. Pathological and morphological effects of *Ustilago tritici* (Pers.) Rostr. on winter wheat. *Dissertation Abstr.* **15**: 681-682.
- O'Kelley, J. C. 1957. Boron effects on growth, oxygen uptake and sugar absorption by germinating pollen. *Am. J. Botany* **44**: 239-244.
- Petri, L. 1914. Studi sulle malattie dell'olivo. V. Ricerche sulla biologia e patologia florale dell'olivo. *Mem. staz. patol. vegetale Roma* pp. 5-64.
- Petri, L. 1927. Formazione prevalente di frutti ipoplastici nell'olivo per causa parasitaria. *Boll. staz. patol. Vegetale* [N.S.] **7**: 447-454.
- Petri, L. 1942. Recenti progressi degli studi sulle malattie dell'olivo. In "Convegno di Studi Olivicoli." Reale Accad. Georgofili, Firenze. 47 pp.
- Ramstad, E., and G. Gjerstad. 1955. The parasitic growth of *Claviceps purpurea* (Fries) Tulasne on Rye and its relation to alkaloid formation. *J. Am. Pharm. Assoc.* **44**: 741-743.
- Russo, F., and P. Spina. 1952. Indagini sulla formazione delle cosiddette pseudo-drupe nell'olivo. *Ann. sper. agrar. (Rome)* [N.S.] **6**: 101-118.
- Sachs, R. M. 1956. Floral initiation in *Cestrum nocturnum*, a long-short day plant. III. The effect of temperature upon long day and short day induction. *Plant Physiol.* **31**: 430-433.
- Salisbury, F. B. 1955. The dual role of auxin in flowering. *Plant Physiol.* **30**: 327-334.
- Salisbury, F. B. 1957. Growth regulators and flowering. I. Survey methods. *Plant Physiol.* **32**: 600-608.



- Savastano, C. 1934. Ricerche fisiologiche sul raggrinzimento delle drupe dell'Olivio. *Boll. staz. patol. vegetale* [N.S.] **14**: 79-116.
- Shirley, H. L. 1929. The influence of light intensity and light quality upon the growth of plants. *Am. J. Botany* **16**: 354-390.
- Smith, H. J., W. J. McIlrath, and L. Bogorad. 1957. Some effects of iron deficiency on flowering of *Xanthium*. *Botan. Gaz.* **118**: 174-179.
- Sommainsi, L. 1954. La concimazione azotata ritardata all'Olivio osservata nel tempo e nello spazio. Parte I. Risultati di cinque anni di esperienze. *Ann. sper. agrar. (Rome)* [N.S.] **8**: 1887-1927.
- Sommainsi, L. 1955. La concimazione azotata ritardata all'Olivio osservata nel tempo e nello spazio. Parte II. Risultati del VI anno di sperimentazione. *Ann. sper. agrar. (Rome)* [N.S.] **9**: 21-43.
- Spencer, J. L., and H. E. White. 1951. Anther smut of Carnation. *Phytopathology* **41**: 291-299.
- Stevens, N. E. 1948. Disease damage in clonal and self-pollinated crops. *J. Am. Soc. Agron.* **40**: 841-844.
- Stevens, N. E., and R. B. Wilcox. 1918. Temperature of small fruits when picked. *Plant World* **21**: 176-183.
- Toole, E. H., V. K. Toole, H. A. Borthwick, and S. B. Hendricks. 1955. Interaction of temperature and light in germination of seeds. *Plant Physiol.* **30**: 473-478.
- Tubbs, F. R. 1955. The control of the vegetative growth and reproduction of perennial plants. *Rept. Intern. Hort. Congr. 14th Congr., London* **1**: 39-50.
- Valleau, W. D. 1949. Injury to tobacco leaves from corn pollen. *Kentucky Agr. Expt. Sta. Univ. Kentucky Ann. Rept.* **62**: 6.
- Verkerk, K. 1955. Temperature, light and the tomato. *Mededeel. Landbouwhogeschool Wageningen* **55**: 175-224.
- Viennot-Bourgin, G. 1937. "Les déformations parasitaires provoquées par les Ustilaginées." Le François, Paris. 189 pp.
- Viennot-Bourgin, G. 1953. Étude morphologique de quelques lésions charbonneuses des végétaux. *Proc. Intern. Botan. Congr. 7th Congr. Stockholm, 1950* pp. 701-702.
- Wareing, P. F. 1956. Photoperiodism in woody plants. *Ann. Rev. Plant Physiol.* **7**: 191-214.
- Wassink, E. C., and J. A. J. Stolk. 1956. Effects of light quality on plant growth. *Ann. Rev. Plant Physiol.* **7**: 373-400.
- Weaver, L. O. 1950. Effect of temperature and relative humidity on occurrence of blossom blight of stone fruits. *Phytopathology* **40**: 1136-1153.
- Young, P. A. 1957. Why tomato flowers fail to set fruits. *J. Rio Grande Valley Hort. Soc.* **9**: 103-104.

## CHAPTER 8

# The Host Is Starved

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Nutrition of the host is one of the basic processes that is impaired by disease. Plants are often starved by pathogens. We may say that such plants suffer from hunger.

The plant suffers from hunger when rots destroy portions of the root so that uptake is reduced, when cankers or galls attack the stems so that transport of food is impaired, or when leaf spots eliminate the tissues that manufacture carbohydrates, amino acids, and the like.

The most obvious gross symptom of starvation in the host is stunting and dwarfing. These symptoms result from the action of numerous pathogens of many types. The farmer sees this in terms of low yields,

shriveled grain, and shrunken profits. Starvation of the host by a pathogen may mean starvation of the farmer as well. And if many farmers are starved, the society that they feed may also starve.

In this chapter we shall deal with the biochemical as well as other aspects of the starvation of the cells in the tissues of the wheat leaf that is invaded by *Puccinia graminis*. In 1917, this fungus starved a lot of cells in a lot of wheat leaves in the wheat belt of the United States. This kept the starch from the growing grains, and this, in turn, starved a big nation during a big war. Famine was averted by promulgating wheatless days and by substituting maize for wheat in the diet. Thus, if the diseased plant hungers, the people may hunger.

The most interesting scientific aspects of hunger in the host are concerned with the effects of obligate and semiobligate parasites in susceptible hosts. This chapter will be devoted primarily to examining how these organisms starve their hosts.

The question of hunger will be given synthetic treatment without many bibliographical references. Among the works that I have been able to consult, I have chosen those which appeared to me to be most concerned with this particular theme about which in fact modern literature is not very rich. Many of the problems related to this chapter are also touched on in other chapters of this treatise such as Disease Losses, Growth Is Affected, Water Is Deficient, Respiration of the Host Is Altered, and Toxins.

## I. THE MECHANISM OF NUTRITION

The green, autotrophic plant is capable of assimilating the substances which are necessary to its survival and its development directly in the form of inorganic elements. A continuous flow of water and salts containing nitrogen, phosphorus, potassium, sulfur, calcium, magnesium, etc. ascends through the vascular bundles from the roots to the leaves. Here the first carbohydrates, as well as the organic acids and the amino acids, are formed by photosynthesis. These are partly consumed to produce energy, and partly condensed in different ways to build up pectic and cellulosic substances for the walls, fat and starch reserves, and proteins and nucleoproteins for the protoplasm and the nucleus, the living part of the cell.

This perfect biochemical work is controlled by a large number of enzymes, or rather enzymatic systems, which, under normal conditions, regulate with an accurate and well balanced rhythm the whole chain of the transformations. This varies from moment to moment with the needs, the age, and the specialization of the cell and with the variations of the medium in which it is living. Waste substances are formed which

are eliminated in various ways. In this complicated procedure, the nutrition, with the uptake and organization into vital substances of new mineral elements, permits the repair of the necessary substances and, therefore, the preservation of the vital rhythm. It also permits the growth, the multiplication, and the reproduction of any organism, from the simplest to the most complex.

This recurrent cyclic rhythm is the essence of physiological life.

## II. THE MEANING OF "STARVED HOST"

### A. *Hunger Conceived as the Difficulty or Impossibility of Assimilating Definite Foods in Definite Quantitative Ratios*

When the chain of the reactions which make up physiological life is broken for whatever reason, we have a disease. One of the fundamental aspects of disease is "hunger" which represents an alimentary demand of the protoplasm which cannot be satisfied either quantitatively or qualitatively. It is in this particular sense that we will understand hunger in the course of this chapter; otherwise, it would be a completely natural, if not necessary, phenomenon. The pathological aspect of the problem thus resides in the impossibility to satisfy, or at least to satisfy adequately and in time, a definite need.

Because hunger is characteristic of the living cell, it occurs only in hosts that maintain more or less prolonged relations of compatibility with a parasite. This occurs only in hosts susceptible to maintaining obligate (Uredinales, Erysiphaceae, Peronosporaceae, virus) or semiobligate parasites, i.e., facultative saprophytes (Taphrinales, Phytophthoraceae, Ustilaginales).

The facultative parasites usually kill the cells before they consume them; on the other hand, in the hosts which are resistant to obligate parasites, a few cells die rapidly around the parasite without having experienced hunger.

The type of integral hunger resulting from pathogenic defoliation by facultative parasites or by saprophytes is well known. Examples of this are the grave damage produced by *Cyclonium oleaginum* to the olive tree, by *Venturia* to pear and apple trees, by *Fumago* to olive and citrus trees. According to whether the defoliation is more or less severe and premature, either the crop only, or all the parts of the plant feel its effects in a more or less obvious way. The plant consumes all its reserves in producing new leaves in the shortest possible time. The effects of a severe defoliation in perennial plants may be noted for several years. Severe defoliation corresponds in a way to the removal of the stomach in an animal. This, too, is related to hunger, however no one has ever



dreamt of studying hunger in an animal deprived of its stomach, but rather in one whose stomach does not work normally. So we will not discuss defoliation, however important for the farmer who often sees his crop, and sometimes the very life of his plants, endangered by it.

On the other hand, in the case of tumors produced by *Agrobacterium tumefaciens*, it is not the parasite that starves the plant directly but rather the neoplastic tissue that is the real parasite of the normal tissues. It grows completely at their expense. While the metabolism of the "hyperfed" tumor tissues has been much investigated, it does not seem that the metabolism of the so-called "normal" tissues of the plant itself has been studied. These are, however, the tissues most affected by the lack of nutrient substances, as is obvious from the great reduction in their size.

We do not intend to treat in this chapter problems related to the disturbances of the mineral absorption due to parasitic molds (*Rosellinia*, *Armillaria*, *Thielaviopsis*, *Fusarium*, bacteria, etc.) or to nonparasitic alterations of the absorption apparatus (asphyxia, excess of acidity, excess of salt concentration, etc.). We shall consider them briefly, however, when we discuss the problem of the transport of substances inside the plant.

The title of this chapter is "The Host Is Starved." The discussion will be confined to the "hunger" produced by parasites; the host in fact implies the parasite. We will thus exclude from the discussion diseases due to lack of macro- or microelements in the soil, but we will, however, point to the fact that many of them (Cu, Fe, Zn, Mn, Mg, Mo, B, etc.) constitute, or are in some way an essential part of very important coenzymes. Thus, their total or partial lack can have the gravest consequences for the general metabolism of the cells, and, therefore, for their normal nutritive process.

### B. Some Characteristics of Hunger

The intimate phenomena of hunger are felt at the cellular level: directly in the green assimilating cells, indirectly in non-green cells. We shall study the hunger produced at the level of the living protoplasm, trying to clarify as much as possible its various mechanisms. Food usage proceeds in the fundamental ways described above—that is, absorption, transport, chlorophyllous synthesis and various biosyntheses which are derived from it, respiration as source of energy, and processes of redistribution of the elaborated substances. We shall study the mechanism of hunger in relation to these various aspects.

It is, however, immediately necessary to distinguish two types of hunger: (a) real or direct hunger, that is, the total or partial lack of

nutrient substance, and (b) hunger due to the inability to assimilate. The latter is the most tragic and preoccupying. We shall see later that much of the nutrient material which should migrate into the non-green tissues, is taken up by the parasite in the invaded territory and that the aliquot which is not used by the parasite accumulates around the center of infection because the host is capable of assimilating only a very small part of it.

This is why the real or direct hunger is felt not so much by the green tissues of the leaf which is invaded by the parasite as by those of the stem and especially those of the root, because both live on the material elaborated in the leaves and transported to them.

More generally, we can say that the tissues which are invaded by the parasite suffer mostly from a "hunger arising from a difficulty in assimilating" whereas the tissues which are far from the center of infection suffer rather from a "hunger arising from a lack of nutrient substances." This naturally does not exclude the possibility that there exist, at a distance, also processes of intoxication which make assimilation difficult.

### III. HUNGER MECHANISMS AT THE LEVEL OF THE HOST CELL

According to what has been said above, three hunger mechanisms should be distinguished: (a) a direct mechanism, due to the plundering of nutrient substances by the parasite, (b) an indirect mechanism due to the effects of the intoxication caused by the toxins which are produced by the parasite or by the host as a defensive reaction, (c) an indirect mechanism due to the blocking or slowing down of the transport, that is, the migration and distribution of the nutrient substances to various parts of the plant.

However, it is not easy, nor is it quite correct, to make a distinction between a direct and an indirect mechanism of hunger, because no parasite exists which is not more or less pathogenic; that is, it is not possible to think of the parasite as a simple commensal who would be content to eat its plateful without having first forced in some way the master of the house, who has not invited him, to give it to him while depriving himself of it. And that is why it is not really proper to speak of a "host." We should rather say "the attacked."

The parasite cannot keep everything to itself. Like every other living organism, it has its own exchange processes and must eliminate something. It sometimes uses these waste products of its metabolism to oblige the host to give it food; and thus the big problem of toxins and intoxication automatically appears. Let us consider then that the host does not remain inert, but that it tends to defend itself more or less efficiently

against the aggressor. In so doing, there often arises a formidable biochemical fight with toxic, that is, nonphysiological substances which more or less hinder the metabolism of the host and/or the parasite. It follows that, if theoretically we can distinguish those two modes of generating hunger in the infected host, we must, however, note immediately that they are practically inseparable because they are not two different things, but at most two moments of one and the same phenomenon.

In general, this treatise deals primarily with pathogenism rather than parasitism. This means in general that the word pathogen is preferred to parasite even though most parasites are pathogens.

In this chapter, however, parasite will be used freely. Parasite is a term to describe mainly the food relation between the invader and its host. In this chapter food is, in fact, the major item of concern; therefore, parasite is the term to be used.

Hunger by intoxication can occur by blocking or slowing down of any function taken as a whole (for example, photosynthesis) or of any fundamental enzymatic activity indispensable to the life of the protoplasm. On the other hand, the blocking can be of importance either in itself, or by the unbalance which it causes in the normal activities of the cell. We shall, therefore, try to distinguish the blocking or the slowing down of the functions by themselves from the alteration of the functional equilibria which they generate.

For these reasons, it seems natural to subdivide this question into the four following mechanisms of hunger: (a) alteration of the distributive processes and abnormal consumption of the metabolites, (b) inhibiting action on the production of the metabolites, (c) alteration of the functional ratios, and (d) blocking or malfunctioning of the translocative processes.

#### *A. Alteration of the Distributive Processes of the Metabolites in the Host and Consumption by the Parasite*

At the basis of this mechanism lie the modifications of cellular permeability produced by the parasite through its action on the function of the plasma membrane which regulates to a large extent the diffusion processes of the nutrient substances and their accumulation in the infected zones to the sole advantage of the parasite which uses them for its own development, while of course depriving the host of them. We shall examine those two aspects of the phenomenon separately.

##### *1. Alterations of the Cellular Permeability*

The modification of cellular permeability is extremely important in pathological processes. It is well known that the plasma membrane

displays a special semipermeability which is not only regulated by the physical laws of osmosis, but is strongly influenced by the protoplasmic metabolism. It is so true, that an increase in the intensity of the respiration also generates an increase in the permeability and that the substances which inhibit or slow down the respiration also lower the permeability (Collander, 1957; Dawson and Danielli, 1952). It is known, moreover, that permeability is regulated by enzymes which act in the outermost part of the cytoplasm, the plasma membrane (Rothstein, 1954), which shows a special physical and chemical structure by which it differs from the rest of the cytoplasm. The plasma membrane is formed by intermixed proteins and lipids, and it must be remembered that the modifications of its permeability, whether in a positive or negative sense, depend on the different activity of those enzymes which are stimulated or inhibited by the toxins of the parasite. It can also be thought that these toxins act directly upon the permeability of the plasma membrane.

The now classic research of Thatcher (1939, 1942, 1943) using the protoplasmolytic method, has re-emphasized the close relationship existing between susceptibility and increase in the permeability of the host cells.

He found, in particular, that the mesothetic reaction of Thatcher wheat to *P. graminis tritici* f. 56 is in some way or other related to the modifications of the permeability caused by the fungus. Infection of the resistant type of wheat causes a decrease in the cellular permeability of the host, whereas, increase in the cellular permeability causes an infection of the susceptible type. Thatcher has moreover observed that, by narcotizing Mindum wheat, he could sensitize it to *P. graminis tritici* f. 36 and at the same time increase the cellular permeability of the host. He obtained similar results when studying the attacks of *Botrytis cinerea* and of *Sclerotinia sclerotiorum* on the petiole of the leaves of celery, and also with other diseases.

Gottlieb (1944) used the tracheal fluid, crude sap, of tomato plants infected by *Fusarium bulbigenum* var. *lycopersici* and obtained an increase in the permeability of the medullar cells of the tomato, while the sap extracted from physiologically wilted tomato plants had no effect on the cells themselves.

It was noted by Humphrey and Dufrenoy (1944) that parasitic relationships between oats and *P. coronata* depend on the availability of phosphorus in the intercellular spaces, in favor of the parasite; hence, the probable hypothesis that cellular permeability of the host is increased by rust.

It is sufficient to recall here the investigations of Gäumann and Jaag (1947); Gäumann *et al.* (1952); Linskens (1955); and Zählner (1955)



on the stimulating action on cellular permeability exerted by the purified toxins (fusaric acid, alternaric acid, lycomarasmin, etc.) obtained from cultures of various breeds of pathogenic fungi (especially *Fusarium* and *Alternaria*). These investigations are concerned chiefly with the wilting of the leaves, that is, the loss of water, and, therefore, will be treated more extensively in Chapter 9 of this volume.

From Thatcher's experiments especially, it appears evident that the first act of the parasite is to open the door of the larder. By doing this, it upsets more or less deeply the distributive economy of the nutrient substances. It is the first step toward "hunger" for the host. The osmotic pressure of the parasite, usually appreciably higher than that of the host, wins in this competition, and the parasite appropriates what is necessary to it. On the other hand, the increased cellular permeability prevents or retards the establishment of an osmotic equilibrium which would restrict the flow of metabolites from the host to the parasite.

Because of this, one of the most characteristic aspects of the resistance is, according to Thatcher, the decrease in cellular permeability, which of course dooms the few impermeabilized cells, but promptly cuts down the food supply of the parasite.

## 2. *Accumulation and Consumption of Nutrient Substances in the Infected Zone*

During the first days of incubation, the action of the parasite on the susceptible host is practically always to stimulate the metabolism. We shall see later that this stimulation is not uniform, that it, therefore, generates unbalance between the main functions; but we note, however, that the initial stimulation concerns the evolution and synthesis processes: fragmentation of the vacuoles, evolution of the chondriosomes in plastids, formation of starch in the amyloplasts (Dufrenoy, 1928a, b; 1932), increase in photosynthesis (Montemartini, 1904; Grecusnikov, 1936; Sempio, 1946, 1950a).

We have seen that, at the same time, the permeability of the plasma membrane increases and the parasite, especially fungus, begins to draw to itself from the neighboring zone the substances which it uses to build up its own protoplasm.

Therefore, the impoverishment in substances which are easily assimilated, that is, true hunger proper, starts in the histological ring which surrounds the invaded cells and is made conspicuous by a lighter green halo. With respect to this, it is useful to recall that Mains, as early as 1917, believed that the halos which surround the rust pustules are "starved zones," as a consequence precisely of the removal of nutrient substances from the invaded centers.

The substances which the obligate parasites seem to take up most avidly are the carbohydrates; they are most probably the intermediate transitory compounds which enter the anabolic cycle of photosynthesis, as well as the catabolic cycle of glycolysis and are closely tied to the phosphorylation processes. They could be, for example, glyceraldehyde, phosphoglyceric acid, dihydroxyacetone, oxalacetic acid, or also a pentose such as ribulose phosphate (Benson and Calvin, 1950; Vishniac, 1955).

These compounds, or others which we do not yet know, are evidently indispensable to the metabolism of the obligate parasite, in the proportions and at the time in which they are formed and are rapidly utilized in the autotrophic host. Otherwise, the failures recorded until now in the attempts to cultivate *in vitro* such parasites could not be explained, excepting the positive result obtained recently by Hotson and Cutter (1951) with *Gymnosporangium juniperi-virginianae*, which must, however, be considered as a quite special case.

On the other hand, the possibility of growing the rusts on leaves or fragments of leaves floating in sugar solutions kept in the dark (Mains, 1917; Waters, 1926, 1928; Trelease and Trelease, 1929; Sempio, 1942a) indicates that the life of these parasites is not of necessity related to the luminous phase of the synthetic process, that is, to the fission of water and the fixation of  $\text{CO}_2$ , but rather to that phase of the carbohydrate metabolism which can take place also in the absence of light.

Other investigations have pointed out the fact that darkness, especially in the ultimate phase of the incubation, as well as the removal of  $\text{CO}_2$ , stops the development of the obligate parasites such as rusts, *Oidia*, and *Peronosporaceae* (Gassner, 1927; Gassner and Straib, 1928; Pohjakallio, 1932; Forward, 1932; Sempio, 1938a, 1939).

One of the most accurate and complete studies on the carbohydrate balance in wheat infected by *Erysiphe graminis* is that of Allen (1942). He worked with Marquis and Axminster wheat, measuring with rigorous methods, during the whole period of development of the disease, the values which are more or less directly related to the metabolism of the carbohydrates: intensity of the respiration, respiratory quotient, intensity of the photosynthesis per mole of chlorophyll (ratio of the intensity of the photosynthesis to the chlorophyll content), saccharose, glucose, and starch content of the leaves.

He was thus able to summarize the results of his analyses and of his calculations as shown in Table I.

Several things can be noted. (a) The photosynthesis, initially very high, decreases rapidly until it reaches, on the ninth or tenth day, a very low level. (b) The respiration follows a curve of rapid increase, with

TABLE I

Age of infection in days:	0	2	4	6	7	8	9	10	11	12
Photosynthesis	45.3	43.0	30.2	19.9	14.6	6.7	6.9	4.4	3.8	3.0
Respiration	2.1	3.0	3.4	8.6	8.0	5.4	5.2	5.3	4.0	2.9
Balance	43.2	40.0	26.8	11.3	6.6	1.3	1.7	-0.9	-0.2	-0.1
Carbohydrate	1.7	2.0	5.0	7.8	3.7	2.5	2.2	1.6	1.5	1.2
Carbohydrate available for export	41.5	38.0	21.8	3.5	2.8	-1.2	-0.5	-2.5	-1.7	-1.3

The figures in this table represent *moles of carbon* per unit weight of leaf per day. The first two rows give the amounts of carbon synthesized and respired on successive days after inoculation. The third is the excess of carbon synthesized over that respired. In the fourth row is given the amount of carbon found in the form of carbohydrate. Subtracting this from the amount not respired gives the carbohydrate available for export on any given day, shown in the last row of figures. (From Allen, 1942.)

TABLE II

MEAN YIELD AND WATER REQUIREMENTS OF MARKTON AND VICTORIA OATS  
INFECTED WITH *Puccinia coronata avenae* AT DIFFERENT STAGES OF  
DEVELOPMENT, IN 1930<sup>a</sup>

## A. MARKTON (Susceptible)

Stage at initial infection	Yield <sup>b</sup> of				Water re- quirement (%)
	Grain (%)	Straw (%)	Roots (%)	Total (%)	
Seedling	0.0	35.2	4.4	22.7	390.8
Boot	.0	50.0	12.5	33.1	285.9
Anthesis	54.7	88.0	29.4	72.0	142.3
Dough	97.3	101.1	92.5	99.0	104.1
Check	100.0	100.0	100.0	100.0	100.0

## B. VICTORIA (Resistant)

Seedling	52.5	85.3	45.3	70.5	139.9
Boot	69.2	90.6	55.7	79.3	124.0
Anthesis	81.7	95.9	80.3	89.8	109.8
Dough	98.3	102.7	99.0	101.0	99.0
Check	100.0	100.0	100.0	100.0	100.0

<sup>a</sup> From Murphy, 1935.

<sup>b</sup> Percentage of that of rust-free check.

a maximum corresponding to the sixth or seventh day. Then it decreases slowly. (c) The carbon balance (the difference between the assimilated and the respired CO<sub>2</sub>), which is very high in the first days, drops rapidly thereafter and becomes negative on the tenth day. This is the "hunger

TABLE III  
MEAN YIELD AND WATER REQUIREMENT, PER JAR OF 15 PLANTS, OF PURE-LINE  
SELECTIONS OF MARKTON AND BOND OATS GROWN IN THE GREEN-  
HOUSE AND INFECTED WITH PHYSIOLOGIC FORM 7 OF *Puccinia coronata*  
*avenae* AT DIFFERENT STAGES OF DEVELOPMENT, IN 1933<sup>a</sup>

## A. MARKTON (Susceptible)

Stage at initial infection	Soil moisture (percentage of saturation)	Average weight (in grams)				Average water require- ment
		Grain	Straw	Roots	Total	
Seedling	85	0.5	30.8	3.1	34.4	527
	50	.5	27.7	2.7	30.8	344
Boot	85	5.7	44.6	6.3	56.6	494
	50	4.5	40.4	5.7	50.6	263
Anthesis	85	14.6	60.7	11.0	86.3	335
	50	10.2	47.0	8.7	65.9	196
Check	85	23.8	68.4	14.2	106.4	269
	50	15.7	54.5	11.1	81.3	166

## B. BOND (Nearly immune)

Seedling	85	19.6	59.4	14.4	93.4	302
	50	14.3	41.8	10.5	66.6	227
Boot	85	20.1	61.5	15.1	96.7	296
	50	15.9	45.9	13.3	75.1	220
Anthesis	85	21.8	64.5	15.8	102.1	290
	50	18.0	47.7	14.1	79.8	221
Check	85	22.8	65.6	15.8	104.2	285
	50	18.4	50.4	14.8	83.6	214

<sup>a</sup> From Murphy, 1935.

for carbon" which is deeply felt in the tissues invaded by the parasite, or better, in those immediately adjacent. (d) The sum of the carbohydrates recuperated in the tissues (saccharose + glucose + starch) more or less follows the pace of the respiration with a maximum peak on the sixth day and then a drop to the initial value. (e) The carbohydrates available for export, especially to the stem and the roots, are already very much reduced by the sixth day and from the eighth day on they even show negative values. And this is the mechanism by which hunger extends to the organs of the host which are far from the infected tissues, that is, to the zones of growth of the stem, roots, flowers, and fruits.

At this point, results obtained by Murphy (1935) on the effects of *Puccinia coronata avenae* on the development of the various parts of oat plants belonging to varieties differently resistant to the fungus, should be reported (cf. Tables II and III).



It is obvious that the more intense and precocious the attack, the more severe the losses. But the important point to note is that the worst effects of hunger are felt by the fruits and by the roots: the fruits can even be missing and the roots be so reduced (even to 5 to 10% of their normal size) that they are able to carry out for only a very small part their function of absorption and of mineral nutrition of the plant. A drastic reduction in the size of the plant follows (to one-quarter or one-fifth of the normal size) and a deep modification of the proportions of its various organs ensues. Hunger is extended to the whole plant.

Allen (1942) made other observations which are of importance for us. At the time of the maximum peak in respiration, which coincides with the maximum vegetative development of the parasite, not only are the maximum peaks for the soluble carbohydrates (saccharose and glucose) registered, but unusual granules of starch (detectable with IKI) appear in the chloroplasts of the mesophyll, under or around the *Oidium* colonies, and then later disappear. This means that the parasite draws to itself the major part of the carbohydrates available to the leaf to the extent that, exactly at the moment of maximum consumption of soluble sugars, for a great part respired, the fungus still succeeds in building up transitory reserves of starch which will be hydrolyzed and used by the parasite in the ultimate phase of the disease (development of conidia).

After the starch has disappeared, small green spots appear under the *Oidium* colonies (this is a well-known phenomenon); they are clearly contrasted against the yellowish background of the rest of the leaf lamina. At this point, the carbohydrates in the leaves are completely consumed and none can be produced any more because the chloroplasts are destroyed and those which are formed again do not seem to be efficient. On the other hand, the respiratory quotient drops notably below the normal value; this proves that substances other than carbohydrates are respired, probably fats, and most probably the proteins of the cytoplasm themselves. The cells of the host consume the last reserves and then die; but the obligate parasite has already succeeded in forming and scattering innumerable conidia.

It does not seem to me that an investigation as accurate as this has been carried out on the consumption of nitrogenous substances, and in particular of amino acids. However, there is a second work by Murphy (1936) which reports sufficiently extensive analytical data concerning the effect of *Puccinia coronata avenae* on the chemical composition of oat varieties diversely susceptible to the attack of this fungus. Table IV reproduces these results.

Generally speaking, Table IV shows that the more susceptible the variety, the stronger is the attack, the more the ash and the soluble nitrog-

TABLE IV.  
EFFECT OF CROWN RUST ON YIELD AND COMPOSITION OF GREEN PLANTS OF SUSCEPTIBLE AND  
RESISTANT OAT VARIETIES (GREEN-WEIGHT BASIS)<sup>a</sup>

Variety	Condition of plants	Yield (in grams)	Solids		Ash		Nitrogen			Sugars		Polysac- charides				
			Total solu- ble (%)	Total insol- uble (%)	Total solu- ble (%)	Total insol- uble (%)	Ammo- nia (%)	Am- ide (%)	Nitrate and ni- trite (%)	Total solu- ble (%)	Total insol- uble (%)	Su- crose (%)	Glu- cose (%)	Levu- lose (%)	Dex- trin (%)	Acid hydro- lyz- able (%)
Markton (suscep.) Loggold (suscep.)	Infected	81.07	5.62	13.52	1.54	1.26	0.050	0.047	0.211	0.319	0.297	0.40	0.40	0.03	0.56	2.75
	rust-free	264.08	6.96	10.43	0.81	0.67	0.014	0.011	0.050	0.084	0.236	2.44	1.88	1.16	0.73	2.12
	Infected	89.96	5.25	11.42	1.04	1.07	0.039	0.039	0.230	0.316	0.254	0.42	0.35	0.06	0.64	2.36
	rust-free	247.14	6.32	10.08	0.76	0.82	0.011	0.013	0.055	0.084	0.224	2.55	1.74	1.13	0.81	1.97
Victoria (resist.)	Infected	172.05	5.37	10.74	1.56	0.90	0.013	0.028	0.050	0.185	0.288	0.95	0.45	0.39	0.50	1.99
	rust-free	221.10	6.53	10.13	1.30	0.74	0.011	0.010	0.033	0.088	0.270	2.37	1.34	1.14	0.60	1.90
Bond (nearly immune)	Infected	235.97	5.70	9.20	1.03	0.57	0.011	0.014	0.026	0.105	0.248	2.54	1.41	0.79	0.61	1.87
	rust-free	276.50	6.39	8.85	0.94	0.53	0.011	0.010	0.025	0.067	0.239	3.15	2.02	1.17	0.70	1.81

<sup>a</sup> From Murphy, 1936.

enous compounds (ammoniacal, nitric, and amidic nitrogen) increase in percentage, whereas the insoluble nitrogen (mostly protein) is affected by only very moderate increases. The increase in ash and in soluble nitrogen must not seem surprising because the strong transpiration, stimulated by the attack of the parasite and by the increased cellular permeability, causes the accumulation of these substances which cannot be utilized but for a very small part, because of the scarcity or even the lack of carbohydrates with which to combine. In fact, beside the increase in ash and in nitrogenous compounds, Murphy also recorded a very definite drop in the carbohydrates.

On the other hand, it must be noted, especially for the nitrogenous compounds, that this is an increase in percentage, not a total increase; it has been seen already how a strong reduction in size, weight, and yield can be brought about by a severe parasitic attack.

Caldwell *et al.* (1934) came to similar conclusions by studying the effect of the attack of *P. triticea* on the crop, the physical characteristics, and the chemical composition of the autumnal wheats.

Novikoff (1937), experimenting with the rust of lucern (*Uromyces striatus*), has encountered in the infected plants a notable decrease in the carbohydrates, in protein and nonprotein nitrogen, as well as in cellulose (to which, however, correspond increased proportions of hemicellulose).

In the Golden Rain variety of oats infected by *P. coronifera*, Kokin and Toumarinson (1934) found a decrease in the photosynthesis, in the chlorophyll, soluble carbohydrate, and protein contents which was proportional to the intensity of the attack. Correspondingly, they noted a drop of about 30% in the yield of grain.

With sunflower plants infected by *Puccinia helianthi*, Yarwood and Child (1938) have shown that, whereas the dry weight (per unit of surface) of the infected leaves increases notably with respect to healthy leaves (up to 30 to 40%), the total weight of the diseased plant (excluding the root) is about half that of the healthy plant.

There is thus a sharp contrast between the local effect and the general effect, due to the alteration caused by the parasite in the distribution of the nutrient substances.

Concerning the starving action of viruses, in the sense of subtraction of substances necessary to the metabolism of the host, we have very significant recent data. Wildman *et al.* (1949) proved that the virus protein of tobacco mosaic is synthesized, in the Turkish and Havana varieties, at the expense of the normal nucleoproteins of the host cells. In fact, the increase in virus protein in the infected tissues corresponds

to a parallel decrease in normal nucleoproteins. About the twelfth day following inoculation, some sort of equilibrium is established and no further increase in virus protein can be observed. The results obtained by Commoner and Dietz (1952) are of the same order; they have observed that the period of most intense synthesis of the virus protein of the tobacco mosaic corresponds to the maximum deficiency in nonprotein nitrogen in the tissues. In those cases, the striking pathological fact thus seems to be the appropriation of nutrient substances by the pathogenic agent (virus) and the consequent undernourishment of the host cell, especially in nitrogenous compounds.

Most interesting also in this respect are the results of Fuchs and Rohringer (1955) who recorded the disappearance of various amino acids—principally leucine, histidine, and often also asparagine and isoleucine—from leaves of Marquis and of Vernal wheat inoculated 7 days earlier with *P. graminis tritici*.

An example of disturbed distribution of the metals in the host, due to the chelating action of the toxins produced by the parasite, is given by the studies of Gäumann and his school (Deuel, 1954; Gäumann *et al.*, 1955; Gäumann and Naef-Roth, 1954, 1956) on the nature and the mechanism of the action of the substances produced *in vitro* by *Fusarium lycopersici*, which causes the wilting of the tomato. Kern (1956), in a synthetic review of the work, summarizes this peculiar action in the following manner: "Toxins may also act by formation of chelate complexes with metals. When lycomarasmin (a dipeptide produced by *F. lycopersici*) is applied to tomato cuttings, it forms water-soluble iron complexes in the stem. The complexes are carried into the leaves where part of the iron is liberated again. Lycomarasmin, therefore, causes iron deficiency in the stem and iron excess in the leaves. Application of the equimolar lycomarasmin-iron complex causes a heavier intoxication because additional iron is introduced into the plant; application of the stable equimolar lycomarasmin-copper complex causes much less intoxication because most lycomarasmin molecules are blocked by the copper." It thus seems to consist in a grave disorder in the normal process of the distribution of the iron between the various parts of the plant: the stem suffers a "hunger for iron" because the toxin takes it away from the stem and discharges it into the leaf where, on the contrary, the detrimental effects of its excess are felt.

As a conclusion to this paragraph, we shall recall the recent results obtained by Shaw *et al.* (1954) and Shaw and Samborski (1956a) with radioactive isotopes.

They used  $P^{32}$  in phosphates, and  $C^{14}$  in various sugars, and found that



in the case of obligate parasites (*P. graminis* and *E. graminis* on wheat and on barley, *P. helianthi* on sunflower), the radioactive elements gather for a great part around the infected zones (under the *Oidium* colonies and around the rust pustules), obviously in response to an imperative demand from the parasite. The phenomenon is much more marked in the susceptible hosts than in the resistant hosts. A clear halo of starch around the urediniosori has also been noted.

In the case of facultative parasites (*Botrytis* sp. on bean, *Helminthosporium* on wheat) which normally kill the tissues before invading them, these authors have, on the contrary, observed that the infected zones are less radioactive than the healthy ones, thus confirming that the attraction and accumulation from the noninfected zones to the affected ones can only take place if the invaded cells remain alive and in active metabolism. The results obtained with facultative parasites are completely similar to those obtained with healthy tissues mechanically wounded: the damaged zones are less radioactive than the normal ones. This confirms the fact that hunger, understood as faulty distribution and waste of nutrient substances, can occur only on the condition that the cells remain alive.

Shaw *et al.* (1954, 1956a) have formulated the hypothesis that one or more substances secreted by the parasite diffuse radially into the surrounding zone and that their concentration determines and regulates the more or less pronounced recall of the metabolites into the infected zone. It seems logical to think that this action consists mainly in increasing the cellular permeability.

In a subsequent work, Shaw and Samborski (1956b) reported that, in Little Club and in Kapli wheat infected with *P. graminis* 15B, the accumulation of radioactive glucose is proportional to the increase in respiration, especially so in the susceptible host.

Yarwood (1955) also obtained similar results by working with radioactive sulfur, phosphorus, and carbon on more than 20 complexes constituted for the most part by obligate parasites. By immersing one of the first leaves of a young bean plant in a radioactive solution ( $P^{32}$ ), he observed that the radioactive compounds were attracted in great quantity in that half of the opposite leaf which he had previously infected with *Uromyces appendiculatus*. The quantitative ratio between the radioactivity of the two halves of the leaf (noninfected:infected) was 1:7870 in favor of the infected part. The example is very significant because it shows how powerful an attraction the parasite exerts even at a distance. It is thus clear that an intense and rapid flow of nutrient substances is established toward the infected part even from relatively remote zones which, therefore, remain undernourished and starved.

## B. Inhibiting Action on the Production of Nutrient Substances

### 1. Blocking or Slackening of Photosynthesis

We have already treated this problem when reporting on Allen's experiments (1942). The gradual decrease in photosynthesis from the start to the end of the experiment has been noted from the point of view of the impossibility of compensating adequately the losses, mainly of carbohydrates, due to the suction and to the destruction wrought by the parasite.

We must remark on the fact that as early as 1904, Montemartini had pointed out, for some groups of Uredinales (*Aecidium*), the gradual attenuation of the photosynthesis and the intensification of respiration in diseased leaves. Later, Grecusnikov (1936), experimenting with the complex oats-*Puccinia coronifera* reached similar conclusions: a gradual decrease in the photosynthesis and an increase in the respiration.

Sempio (1946) followed the photosynthesis in the first leaf of young wheat plants heavily infected by *Erysiphe graminis* from the beginning to the end of the development of the infection and made a parallel study on the healthy plant. He noticed that the fixation of  $\text{CO}_2$  decreases, even if irregularly as we shall see later, down to 30 to 40% of the normal values, while the respiration is extremely high.

Kuprevicz (1947) reports, for various rusts, a more or less notable reduction in the photosynthesis, usually around 50% of the normal value. This is always related to a decrease in the chlorophyll content. He observed that the reductions from normal values were greater in the morning than in the evening. An extensive and rapid destruction of chlorophyll has been observed also by Braun (1937) in the chlorotic halo which surrounds the points of infection of tobacco by *Pseudomonas tabaci*, the agent of wildfire.

The depression or even the blocking of the photosynthesis in the diseased plants is thus a general phenomenon. According to Allen (1942), it is closely related to two causes: (a) to the destruction of the chlorophyll, as the chlorotic color of the diseased tissue clearly shows, and (b) to the decreased efficiency of the photosynthesis per mole of chlorophyll, especially when the disease is in an advanced stage.

The importance of the total or partial blocking of the photosynthesis in the nutritive economy of an autotrophic plant needs no further comment.

### 2. Action of Antimetabolites and Blocking of Enzymes

The best known and most characteristic case of this particular type of "hunger" is that illustrated in the studies by Braun (1950, 1955) and

Woolley *et al.* (1952, 1955) on the toxin of wildfire of tobacco. They have isolated and purified from a culture of *Pseudomonas tabaci* a toxin which reproduces exactly the symptoms of the disease on the tobacco leaves, that is, the typical chlorotic halo. The toxin has a structure similar to that of methionine, and behaves as an antimetabolite for methionine. These authors succeeded in obtaining the evidence of this competitive action only in liquid cultures of *Chlorella* by adding to the culture a dose of methionine proportional to the dose of purified toxin, the toxic effect of the latter disappears.

They have, however, not been able until now to reproduce the phenomenon on the tobacco leaves, with which they succeeded only in obtaining the reproduction of the symptoms by inoculating the purified toxin. If it is possible to prove, as seems probable, that the toxin of wildfire behaves as an antimetabolite of methionine also in tobacco, we shall have a classic example of "hunger through competitive action." It is well known that methionine is one of the most important amino acids of the protein metabolism. The competitive action of the antimetabolite (the toxin) would consist in appropriating the enzymatic system necessary for the biosynthesis or the subsequent metabolism of methionine, thus creating a "hunger for methionine."

Now that many investigators have focused their attention on the nature and the mechanism of the action of the toxins and are working systematically in this field, it is easy to forecast that they will discover many toxic actions of the type already described, consisting precisely in the inhibition of the biosynthesis or the later utilization of metabolites which are fundamental in the vital economy of the host, through blocking and competitive appropriation of enzymatic systems. Thus, they will clarify many types of starvation.

A case which is as well known in the literature, but completely reversed—insofar as it is a case of "hunger in the parasite" instead of the host—and which is reported here only by analogy, is that discovered by Hassebrauk (1952) and later confirmed by various authors, on the antagonistic action of the sulfa drugs as regards the utilization of PABA by the rusts of cereals. The sulfa drugs, administered through the roots or by spraying the foliage of the plants, inhibit the development of the rusts; but the fungistatic effect of the sulfa drugs disappears and the rust develops if an adequate dose of PABA is administered to the plant. The biochemical process is the same, but in this case it is the parasite which is starved.

During the process of infection, amino acids which are most important for the metabolism of the plant may disappear. We have already recalled the recent chromatographic investigations of Fuchs and Roh-

ringer (1955) on young plants of Marquis and of Vernal wheat infected by *P. graminis tritici* 126A. The disappearance (in both varieties of wheat) of various substances which react positively with ninhydrin, and among these mostly histidine, leucine, and often isoleucine and asparagine, seems to indicate, rather than a direct utilization of these substances by the parasite, a different orientation of the nitrogen metabolism of the plant under the stimulation of the toxins or the enzymes produced by the parasite.

It is logical to think this, especially in view of other recent studies which have confirmed, with the help of refined cytochemical techniques, the production of enzymes by the haustoria of fungi. Atkinson and Shaw (1955) have demonstrated precisely the presence of notable concentrations of acid phosphatase inside and around the haustoria of *Erysiphe graminis* in the epidermal cells of barley. The authors think that acid phosphatase plays a fundamental role in the transfer process of the metabolites, especially carbohydrates, from the cytoplasm of the host to that of the parasite; but it must be admitted that these enzymes have a deep influence on the whole enzymatic frame of the host cell. Besides, the cases illustrating the stimulating action of the parasite on various enzymatic complexes of the host, especially of the group of the oxidases, are now sufficiently numerous.

### C. Alterations of the Functional Relations

In a few works, Sempio (1942c, 1946, 1950a, b) and Ottolenghi *et al.* (1953) have pointed out the importance of the intensity ratios between the various functions of the diseased plant, not only as one of the elements which enable us to understand better the pathological process, but also as one of the many mechanisms which the plant uses to defend itself against the attacks of the parasite. Of course, we shall see here only those aspects of the problem which are related to the question of starvation of the host. It will be necessary, however, to go somewhat deeply into a few questions which will be treated more extensively in Chapter 10 of this volume.

#### 1. Alteration of the Ratio Photosynthesis:Respiration

Montemartini (1904) and Grecusnikov (1936) have encountered a short but sensible excitation of the photosynthesis at the beginning of the infection in plants attacked by rusts (the former, *Aecidium*, the latter, *Puccinia conifera*). The photosynthetic activity then drops rapidly, while the respiration tends to increase more and more as the infection proceeds.

Allen's (1942) graphs of the metabolism of powdery mildew of wheat,



where he compares directly the course of photosynthesis in diseased plants and in healthy ones (Figs. 7, 10, 13), show clearly that in the 3 or 4 first days of incubation, the photosynthesis of the diseased plant is excited with respect to the normal value. Then it decreases rather rapidly, according to the degree of infection, to values about one-third to one-fourth of the normal ones. The more severe the attack, the more rapid and intense is the decrease. Other graphs show that respiration increases rapidly in diseased plants to a maximum around the eighth day. After that, respiration decreases rapidly.

Allen has purposely carried out an investigation in an absolute sense which would enable him to construct the carbon balance. Sempio (1946 and 1950a), on the contrary, working also with powdery mildew on

TABLE V.  
PHOTOSYNTHETIC ACTIVITY OF LEAVES OF VIRGILIO WHEAT  
INFECTED BY *Oidium moniloides*<sup>a</sup>

Number of days after inoculation	Weather	Temperature (°C).	Duration of experiment (in hours)	Milligrams CO <sub>2</sub> fixed by 1 gm. dry matter in 10 liters of air <sup>b</sup>		CO <sub>2</sub> fixation by infected plants if value of 100 is given to healthy plants
				Healthy leaves	Infected leaves	
First leaf						
2	Clear	16-22	6.30	24.07	41.26	171.2
4 <sup>c</sup>	Cloudy	15-16	6.30	20.65	24.30	117.7
6 <sup>d</sup>	Clear	13-17	7.30	27.05	18.07	66.7
8 <sup>e</sup>	Clear	14-20	8.00	19.30	26.76	138.7
11 <sup>f</sup>	Cloudy	16-20	6.30	17.88	22.03	123.2
14	Clear	16-23	4.15	20.07	9.19	45.7
First and second leaves						
1	Clear	21-27	6.30	5.96	8.88	149.0
3 <sup>d</sup>	Clear	19-29	7.00	8.47	7.94	93.7
5 <sup>e</sup>	Cloudy	17-20	7.00	7.08	13.76	194.4
7 <sup>f</sup>	Clear	14-26	7.15	7.56	11.10	146.8
10	Cloudy	17-21	6.15	30.90	29.30	94.8
12	Clear	12-22	7.45	22.55	3.22	14.3

<sup>a</sup> From Sempio, 1946, 1950a.

<sup>b</sup> In all experiments with only the first leaf and in the experiments at 10 and 12 days after inoculation of the first and second leaves, the atmosphere was enriched with CO<sub>2</sub>. This explains the high values for fixation of CO<sub>2</sub>.

<sup>c</sup> Haustoria.

<sup>d</sup> Mycelium and haustoria well developed.

<sup>e</sup> Conidiophores and conidia formed.

<sup>f</sup> Conidia abundant, disease attack severe.

wheat, collected relative data systematically keeping in mind an ultimate comparison of the functions of diseased and healthy plants of the same age, variety, and cultural conditions. He was thus able to express constantly the data obtained with the diseased plant, during the complete cycle of the disease, as percentages of those obtained with the healthy plant. This permits a demonstration of the extraordinary imbalance which the pathogen produces in the normal functional relations of the host plant, whether in the incubation cycle or during the period when there are exterior manifestations of the disease. In Tables V and VI, the experimental data on the photosynthesis and the respiration in relation with the degree of development of the disease are reported.

From these experiments, and especially from the percentile values (last column), it appears that the fixation of carbon is markedly greater than normal during the first 2 to 3 days of the incubation, that is, at the

TABLE VI.  
RESPIRATION OF LEAVES OF VIRGILIO WHEAT INFECTED BY *Oidium moniloides*<sup>a</sup>

Number of days after inoculation	Temperature (°C.)	Ratio of fresh weight to dry weight (mean value in %)		Average value <sup>b</sup> of QO <sub>2</sub>		Respiration of infected plants if value of 100 is given to healthy plants
		Healthy	Infected	Healthy	Infected	
First leaf						
2	24.6	9.0	9.2	1.73	2.06	119.1
4 <sup>c</sup>	23.1	9.7	9.5	0.90	1.18	131.1
6 <sup>d</sup>	23.5	8.7	9.6	1.15	2.04	178.2
8 <sup>e</sup>	24.5	8.7	9.3	1.20	3.40	283.3
11 <sup>f</sup>	24.6	8.7	10.5	0.86	3.73	433.7
14	23.8	9.0	10.2	0.81	3.09	382.1
Second leaf						
1	24.9	11.9	11.3	1.65	2.07	124.8
3 <sup>d</sup>	25.3	10.9	11.5	1.61	2.97	184.6
5 <sup>e</sup>	23.8	9.5	11.2	1.23	3.94	319.7
7 <sup>f</sup>	24.4	9.8	10.5	1.29	4.00	309.7
10	24.4	10.7	11.0	1.25	3.62	290.0
12	24.4	10.0	11.8	1.22	3.18	260.0

<sup>a</sup> From Sempio, 1946, 1950a.

<sup>b</sup> Respiration was determined by the Warburg micromanometric method. QO<sub>2</sub> = mm.<sup>3</sup> of O<sub>2</sub> absorbed by 1 mg. dry matter in 1 hr.

<sup>c</sup> Haustoria.

<sup>d</sup> Numerous haustoria and mycelium well developed.

<sup>e</sup> Conidiophores and conidia formed.

<sup>f</sup> Conidia abundant, disease attack severe.

moment of the implantation of the first haustoria; then it decreases below the normal value and increases again above it at the time of the differentiation of the conidiophores and conidia; at the end, it decreases definitely to very low values.

Respiration, on the contrary, at first only slightly excited, rises rapidly at the time of the differentiation of the conidiophores, with a maximum peak 3 to 4 times the normal value; then it decreases rather slowly, remaining however, at least within the limits of the experiment, much higher than is normal.

If we now calculate the ratio of the percentile values (the normal values being taken as 1) of photosynthesis to respiration, we note that, at the beginning, this ratio is markedly higher than 1 ( $171.2:119.1 = 1.43$ ), whereas it later drops gradually to values near 0 ( $45.7:382.1 = 0.12$ ).

The marked initial stimulation of photosynthesis during the first phase of the infection—for which the ratio of photosynthesis to respiration rises markedly above the normal value—is considered by Sempio (1946, 1950a, b) as a defensive reaction of the host, because the plants, which are put in the dark during the first phase of the incubation, are usually more heavily affected than the controls which are left in normal light (Sempio, 1939).

Sempio believes that this imbalance between anabolism and catabolism, that is, between synthesis and breakdown, or again between endothermic and exothermic reaction, has consequences, not only for the building up of material (breakdown without reconstruction) but also, and above all, in the field of energy. As far as we can understand, the parasite stimulates precisely the respiratory processes so as to obtain the energy necessary to the synthesis of its own protoplasm, and we shall see this better in what follows.

On the other hand, it seems obvious that the host is not able to utilize physiologically the excess calories from respiration because, as Allen (1942), Shaw *et al.* (1954) and Shaw and Samborski (1956a) have shown, starch accumulates around the infected zones while the invaded cells are already in a state of irreversible undernourishment. In all probability, the accumulation of starch is also related to the disappearance of some fundamental amino acids, as the work by Fuchs and Rohringer (1955) has shown. According to this work, the fraction of sugars which should constitute the substrate for the building up of amino acids and then of proteins, if not utilized, would accumulate in the form of reserve starch which the parasite would later use for its own needs in energy.

Metabolism is an uninterrupted chain of reactions closely interdependent; therefore, the "hunger for amino acids" can have as a conse-

quence the nonutilization and the accumulation of carbohydrates flocked from the surrounding zones, and vice versa. In the histological territory invaded by the parasite, the type of "hunger amidst abundance" which we mentioned at the beginning of this chapter is thus verified.

## 2. Alteration of the Ratio Glycolysis:Respiration

This problem has been a subject of interest and discussion in recent years, especially in the numerous investigations carried out to discover the physiological reasons and the biochemical mechanisms of the great increase in respiration during pathogenesis.

Sempio (1942c) has noticed that during the period of incubation of the bean rust (*Uromyces appendiculatus*), the respiration rises markedly above the normal value (2 to 3 times), while the glycolysis (evolution of  $\text{CO}_2$  in anaerobiosis) shows only slight increases or even decreases. Similar data, if less clear cut, were found with the disease of lettuce caused by *Bremia lactucae*.

With the powdery mildew of wheat caused by *E. graminis* (Sempio, 1946), the experimental results are even more significant: respiration rises rapidly to 3 to 4 times the normal value, while glycolysis, after a slight initial stimulation, drops gradually to rather low values in comparison with the healthy plant (around 50%). In a successive work (1950a) Sempio emphasized this striking displacement of the functional equilibria in the infected plant, noting that at the end of the period of incubation, the contrast between the increase in respiration and the decrease in glycolysis is very strong in comparison with the healthy plant.

Table VII reports on the glycolysis tests made on wheat attacked by *Oidium*. The comparison of these data with those obtained for respiration (Table VI) shows the marked imbalances which, together with those already reported on the ratio of photosynthesis to respiration, give a picture of the functional disturbances produced by the parasite in the tissues of the host, and thus also of the possible immediate repercussions on the nutritive processes.

Allen (1953, 1954), taking Sempio's data as a starting point, and assuming that the respiratory quotient is practically equal to 1, noticed that from the ratio of the  $\text{CO}_2$  evolved in anaerobiosis and in aerobiosis

$$Q_{\text{CO}_2}^{\text{N}}:Q_{\text{CO}_2}$$

the inhibition of the Pasteur effect appeared evident. In fact, from the examination of the values obtained with Virgilio wheat it can be seen that, usually around the sixth day after inoculation—that is, when the differentiation of the conidiophores starts—the ratio



$$Q_{CO_2}^N : Q_{CO_2}$$

drops below 0.33, which is the minimum value for which the Pasteur effect still operates. In healthy leaves, on the contrary, this effect is always largely secured.

Allen presented the possibility that toxins diffusing into the tissues of the host inhibit the Pasteur effect by uncoupling the processes of oxidation and phosphorylation, thus permitting an uncontrolled increase in the respiration. It is known in fact that in the normal respiration, the oxidative processes are always coupled with those of the phosphorylation, so that the latter constitute a sort of control and limitation of the former, that is, a physiologically economical limitation.

The toxin of the pathogen acts, perhaps, in the same way as some stimulants of the respiration such as, for example, DNP in small doses ( $10^{-5} M$ ). Thus, it would have an uncoupling action on the two activities

TABLE VII.  
GLYCOLYSIS IN LEAVES OF VIRGILIO AND MENTANA WHEAT  
INFECTED BY *Oidium moniloides*<sup>a</sup>

Number of days after inoculation	Tempera- ture (°C.)	Ratio of fresh weight to dry weight (mean value in %)		Average value <sup>b</sup> for Q <sub>CO<sub>2</sub></sub> <sup>N</sup>		Glycolysis of infected plants if value of 100 is given to healthy plants
		Healthy	Infected	Healthy	Infected	
Virgilio—second leaf						
2	24.5	11.7	11.2	0.95	1.17	123.0
4 <sup>c</sup>	24.5	10.6	10.8	1.07	1.16	108.7
6 <sup>d</sup>	24.4	11.8	11.7	1.05	0.83	79.3
8 <sup>e</sup>	24.4	10.7	12.3	0.86	0.60	70.6
11 <sup>f</sup>	24.5	11.1	11.4	1.04	0.65	62.1
14	24.4	11.4	13.5	0.80	0.45	55.9
Mentana—first leaf						
1	25.3	8.93	9.38	0.66	0.82	123.9
2	24.8	9.03	9.12	0.68	0.73	107.5
4 <sup>c</sup>	25.0	8.33	9.23	0.74	0.72	95.9
6 <sup>d</sup>	25.3	8.48	8.90	0.60	0.63	106.6
8 <sup>e</sup>	25.3	8.50	9.19	0.74	0.64	87.0
11 <sup>f</sup>	25.1	8.70	9.93	0.85	0.58	68.2

<sup>a</sup> From Sempio, 1946, 1950a.

<sup>b</sup> Glycolysis was determined by the Warburg micromanometric method.  $Q_{CO_2}^N$  = mm.<sup>3</sup> of carbon dioxide emitted in 1 hr. by mg. dry matter in an atmosphere of nitrogen.

<sup>c</sup> Haustoria.

<sup>d</sup> Well-developed mycelium.

<sup>e</sup> Conidiophores and conidia formed.

<sup>f</sup> Conidia abundant, disease attack severe.

and would remove the natural restraint from the oxidative processes of the host. This would result in the exceptional and pathological consumption of  $O_2$ , and consequently in the blocking of the Pasteur effect which consists precisely in stopping or slowing down the glycolysis in the presence of  $O_2$ . The accumulation of inorganic phosphorus encountered by various authors in infected tissues with high respiration would also be in favor of this hypothesis.

Allen's ideas are usually shared by the various authors who have studied this problem in the last few years. I will mention the following in particular.

Farkas and Király (1955) worked with the rust and the powdery mildew of wheat, caused by *P. graminis* and *E. graminis*, respectively. They drew the conclusion that both parasites induce an aerobic fermentation in the infected tissues and that the Pasteur effect is blocked. Millerd and Scott (1956) suggest that the strong increase in respiration of barley infected with *E. graminis* is due to the uncoupling of the oxidative phase from the phosphorylative phase by the action of substances which are formed during pathogenesis. Uritani and co-workers (1954, 1955) and Akazawa and Uritani (1955a, b) following numerous studies on the black rot of sweet potato caused by *Ceratostomella fimbriata*, considered as very likely an uncoupling action of ipomeamarone (a toxin produced by the fungus in the culture) but believed that this action is only partially responsible for the increase in respiration encountered in the infected tubers. It should be noted, however, that Uritani *et al.* (1954 and 1955) worked with a facultative parasite of non-green organs; but, as also Farkas remarks very appropriately (1957), it is dangerous to draw parallels between the behavior of obligate and facultative parasites, especially in the field of these most delicate metabolisms, because their needs are very different. It is enough to recall that, while in the obligate parasite the increase in the respiration of the host is always practically a *sine qua non* condition for the subsequent development of the parasite, and thus is a characteristic of the susceptible species, in the case of many facultative parasites, the resistance of the host on the contrary is associated with a high respiratory rhythm (Fuchs and Kotte, 1954; Walker and Stahmann, 1955; Rubin *et al.*, 1955). Shaw and Samborski (1957) have also recently investigated the mechanism of the high respiration in wheat infected by *P. graminis* 15B and they observed that the ratio  $NR:OR$  ( $CO_2$  evolved in anaerobiosis and in aerobiosis) decreases to values of 0.2 to 0.3, for which the Pasteur effect is considered to be totally blocked and for which, therefore, the degradation of the carbohydrates occurs.

However, contradictory opinions are not lacking on these points. Daly and Sayre (1957), working with the rust of safflower (*P. carthami*),

believe that an uncoupling action cannot be held responsible for the increase in respiration and for the blockade in Pasteur effect which they also found in the infected plants at the time of the formation of the sori. Their disagreement with the uncoupling theory arose because they recorded simultaneously notable increases in the elongation and in the wet and dry weight of the infected hypocotyls in comparison with the healthy ones. They hold that where such evident processes of protein synthesis exist, there is no place for an uncoupling action. Therefore, the increase in respiration and the blocking of the Pasteur effect must be attributed to other mechanisms which they believe to be hormonal in nature.

It should be noted in connection with these criticisms by Daly and Sayre, that the disease which they studied can be considered, under certain aspects, as a borderline case between parasitism and mutual symbiosis, at least when limited to the infected tissues. In fact, the cases where an obligate parasite causes such high increases are rather rare. On the other hand, the authors do not report the weight of the total plant, with the leaves and the roots, but only that of the hypocotylous region. It is convenient, however, to admit that while in certain cases the strong increase in respiration can be related to an uncoupling action and thus depend on the regulating systems of the metabolism, in other cases it can depend on hormonal actions or on other mechanisms.

As can be seen, the problem is still open to investigation and to discussion and will be much more extensively and competently treated by others in Chapter 10. It has been thought opportune to explain the question briefly here only because the blocking of the Pasteur effect and the possible uncoupling effect are closely related to the subject of this chapter. In fact, the blocking of the Pasteur effect practically implies the impossibility of partial resynthesis of the carbohydrates which is characteristic of the normal aerobic process, and therefore also implies the destruction of this valuable plastic material which is to be used in building up the protoplasm. On the other hand, a consequence of the uncoupling action is the useless dispersion of energy which, in the normal biological economy, is destined to the syntheses necessary to the host cell. A waste, and therefore a "hunger for energy" is thus generated in the susceptible host while the parasite seems to be able to use it for its own development.

### *3. The Host Cell Burns Slowly*

In the case of obligate parasitism, the experiments of the various authors seem to be concordant in some fundamental lines summarized as follows. The parasite starts to differentiate the conidiophores and the conidia usually around the eighth or tenth day of incubation under

normal light and temperature conditions. At that time, the metabolic pattern of the host is the following: photosynthesis is partially blocked, and respiration is at its highest; the Pasteur effect is completely blocked, and an uncoupling action of toxic origin seems to arise and prevent an efficient utilization of energy. So in the end, the sources of supply are cut off and the provisions become badly dispersed. The nutritive balance of the host could not be more tragic and bankrupt; in fact, it always ends with the death of a more or less wide histological zone. By the time this occurs, however, the parasite has already completed its cycle.

#### D. *The Blocking of the Transport*

We have already mentioned the anomalies which the disease generates in the distribution of the nutrient substances in the tissues of the host altering the cellular permeability and allowing an abnormal concentration of nutrients in the infected zone at the expense of the neighboring tissues or even of those far removed from the point of infection.

We must now speak briefly of the effects of the disease on the efficiency of the main routes along which the nutrient substances move to reach the place of consumption or of storage, that is, of the functional alteration of the conductive system. This is valid mostly for those tissues and those organs which are not themselves productive but must be supplied in time with substances elaborated or stored elsewhere.

On the transport of water and mineral salts there is not much to be said because the question will be treated to a large extent in the next chapter on the "thirst" in plants, partly also in the chapter on toxins (Chapter 9 of Volume II).

From our point of view, the question is of interest because the ascending sap carries all the mineral elements necessary for biosynthesis, with the exception of carbon. Therefore, all of the pathogens which inhibit or hinder the absorption by the roots or the transport by the ascending sap, generate a hunger for mineral substances, especially nitrogen, phosphorus, and potassium, which are necessary in large quantities.

The tracheomycoses produced by *Fusarium* and by *Verticillium* have been much studied, and in particular the wilting of the tomato caused by *F. oxysporum* f. *lycopersici*. In this case, however, the authors usually agree that, apart from the blocking of the xylem ducts, the toxins or enzymes carried along with the flow of sap are also, and above all, responsible for wilting. Which toxins and/or enzymes are responsible for the wilting and what is the mechanism of their action, is still a matter for controversy.

Gäumann and his school claim that it is mostly lycomarasmin and



fusaric acid extracted from the culture of the fungus and purified, whereas Dimond (1955), as well as Walker and Stahmann (1955), believe some pectolytic enzymes, such as polygalacturonase (PG) and pectinmethylesterase (PME) to be mainly responsible for the wilting. Besides, the discussion on the "vivotoxins," raised by Dimond and Waggoner (1953, 1955), requires great consideration.

Anyway, this question will be treated in another chapter. It is enough for us to recall that the transport of the salts is partly hindered by mechanical occlusion of the ducts, partly altered by the chelating action of the toxins (we have already mentioned the action exerted by lycoramasmin on iron), which are believed to block certain elements during the transport and to alter their distribution.

On the other hand, the occlusion of the ducts caused by the zoogloeae of *Erwinia tracheiphila*, which causes a wilt in the Cucurbitaceae, and of *Phytophthora blight*, agent of the bacteriosis of maize (Harris, 1940), is of more closely, if not exclusively, mechanical nature.

It should be noted that this scarcity of mineral elements is usually not felt as real hunger because the cells die first either of thirst or of intoxication. It can thus be explained that the experimenters have often recorded relative increases (with respect to the dry weight) in mineral substances in wilted plants as compared with normal plants.

The disturbances caused by the pathogens to the other great route of transport, the phloem, even if the cases are less numerous, are undoubtedly more closely related to the problem treated here. We shall mention only a few aspects of the leptonecrosis of the potato, produced by the virus of leaf roll, one of the most investigated diseases.

Since Quanjér *et al.* (1916) noticed that the necrosis of the phloem is the most important and constant histological characteristic of leaf roll of the potato, various authors have studied the problem from the histological and biochemical points of view.

It has been seen that, above all, the primary phloem is deeply altered, as is shown by the yellow-reddish color it assumes. The walls of the cells become thick and partly lignified, and this process tends to close completely the lumen of the tubes. But even before the phenomenon becomes perceptible, it has been observed that the sieve-like tubes lose their function to a large extent. Therefore, the transport of the carbohydrates from the leaves cannot take place or is very much hindered.

Whitehead (1927) observed that the migration of the carbohydrates from the leaves to the tubers during the night is so much reduced in the infected plants, that on the next morning, when photosynthesis starts again, the leaves are still full of starch. Thus, the starch tends to accumulate in the leaves rather than in the tubers.

Further precisions on the metabolism and transport of carbohydrates and of nitrogenous compounds in the infected potato have been given by the investigations of Barton-Wright and McBain (1932). They found that the carbohydrates, not being able to migrate from the diseased leaves, are submitted to some sort of cyclic metabolism according to this scheme: starch-hexose-saccharose-starch. They observed that, despite the decrease in photosynthesis and the notable increase in respiration, the starch continues to accumulate in the leaves; this confirms the fact that very little carbohydrate succeeds in migrating to the reserve tissues and this is why the form of cyclic metabolism mentioned above is established. They have, moreover, noted that in the healthy plants, first a hexose, then saccharose, which is the carbohydrate normally destined to migrate to the tuber through the phloem, are formed by photosynthesis. They have never found, on the contrary, any saccharose in the petiole of the leaves of diseased plants; this means, according to the authors, that the little carbohydrate which succeeds in reaching the tubers does not migrate as saccharose through the ordinary route (the phloem), but as hexose through the parenchyma (ground tissue). The consequence of this altered mechanism of transport is that the varieties which are very susceptible to the virus of leaf roll, such as for example the President variety, only give a tuber crop of one-tenth or one-twelfth of the normal crop, while the leaves change color, often become fragile, and roll up, because they are not able to digest the excess carbohydrate.

According to Murphy (1923), it is the spongy tissue of the leaf which, because of its looser structure, is best suited to the storage of the excess starch. It expands under the pressure of the filling, while the more rigid palisade layer remains unextended, and this leads to the rolling up of the leaves.

It is interesting to note that the infected tubers give up very slowly to the new shoots the substances which they have succeeded in storing. McLean (1926a, b) has in fact observed that the diseased tubers remain hard and turgid even after germination, while the healthy ones are already emptied, flaccid, and contracted because they have given all their contents to the new young plants. Thus, the shoots of the infected tubers grow extremely thin and the effects of hunger become more cruel and irreparable from year to year.

#### IV. THE CONSEQUENCES FOR THE HOST OF THE VARIABLE NUTRITIVE NEEDS OF THE PARASITE DURING THE PERIOD OF INCUBATION

This section refers to the diseases caused by obligate parasites or parasites which establish in one way or other a prolonged symbiosis with the host.

We have already seen that all parasites, but especially the obligate parasites, have very high needs in carbohydrates, and that in all probability the intermediate metabolites which arise from photosynthesis or from glycolysis are their preferred substrate.

It must, however, be underlined here that these demands are not the same for the total period of the symbiosis of the two elements of the complex. They vary a great deal, mostly during the incubation period.

For what can be understood from indirect experiments, the demand for carbohydrates increases as the incubation proceeds, but becomes suddenly stronger at the moment of the formation of the conidiophores and conidia. Waters (1926) has observed that the most severe infections on bean leaves infected by *U. appendiculatus* appear on leaves which, kept floating on water until the sixth day after the inoculation, are then transferred into a sugar solution. Forward (1932) inoculated young wheat plants with *P. graminis tritici* f. 21. On the sixth or seventh day after inoculation, she placed them in the dark and observed that they became covered with spots of hypersensitivity rather than with urediniosori of the susceptible type. This is another proof of the imperative needs of the parasite for carbohydrate at that particular moment.

But the most systematic research on this question has been carried out by Sempio (1938a, 1939, 1942b). He investigated the behavior of some complexes (wheat-*Erysiphe graminis*, wheat-*P. trititcina*, bean-*U. appendiculatus*, lettuce-*Bremia lactucae*, radish-*Albugo candida*) toward the most important external factors (temperature, light, humidity, pressure, CO<sub>2</sub>, O<sub>2</sub>, ultraviolet) during the period of incubation of the disease.

By standardizing this period to an average duration of about 9 to 10 days, he could divide it into three phases of about three days each: phase I, from the moment of contagion to the implantation of the parasite in the tissue of the host (haustoria formed); phase II, expansion of the parasite into the tissues; phase III, differentiation of the conidial apparatus (conidiophores or sori). These three phases show a different behavior toward the main external factors, and especially toward light and temperature. He observed, for example, that during the first phase, the darkness or a striking decrease in the intensity of light usually stimulates the development of the disease, whereas during the third phase, it results in a regression in the disease itself. In the same way, relatively high temperatures are borne much better in the first two phases than in the third one.

Hassebrauk (1940) has also obtained similar results on wheat varieties which are moderately resistant to *P. trititcina*.

Generally speaking, it has been found that phase III is the most

critical, not only for the parasite, but also for the host, or better, it is critical for the complex host-parasite taken as a biological entity. This proves that the host is then exhausted from hunger and has, therefore, partially or totally lost its normal capacity to resist the unfavorable conditions of the medium (prolonged darkness, high temperatures, etc.).

A typical case of this critical state is shown by the Gotta lettuce infected by *Bremia*. The infected young plants resist the effects of darkness very well during the first 6 days of the incubation, that is, during phases I plus II, whereas they become very flaccid without any possibility of recovery after only 3 days of darkness in the third phase (Sempio, 1938b). When the complex wheat-*P. triticina* is put in the dark during the third phase (from the beginning of the seventh day until the end of the eleventh), a strong yellowing of the leaves occurs. The leaves, however, succeed, after a few days of exposure to light, in regaining a practically normal color, while the urediniosori, which began to be formed at the time of the darkening, are completely aborted.

These results prove that, at the time of the differentiation of the propagules (conidiophores, sori, conidia), the parasite demands from the plant its last nutritive reserves, especially of carbohydrates. At this stage a strong competition arises between the two organisms. If at this critical moment the road to carbohydrate synthesis is cut by removing the light, two things can happen: (a) if the plant still has some useful reserves, if it has rather hard and dry leaves, then it succeeds in overcoming the competition of the parasite in the dark (case of the complex wheat-*Puccinia*); (b) if the plant is at the end of its reserves, has tender and aqueous tissues, then it dies together with the parasite (case of the complex lettuce-*Bremia*).

This interpretation of the experimental results seems to be supported also by the metabolism experiments reported above. See in particular Table V (page 296) relative to the photosynthetic activity of the complex wheat-*Erysiphe*. It can be noted that, comparatively to the healthy plant, the photosynthesis of the diseased plant is more intense during the first 2 to 3 days, then decreases below the normal value, then rises again well above the normal value on the sixth to eighth day, that is, during the third phase of incubation. Similar results (not published) have been repeatedly obtained also with the complex bean-*Uromyces*. In the very simple experimental conditions used by Sempio and under a continuous rigorous comparison with the healthy plant, this revival of photosynthesis (greater fixation of  $\text{CO}_2$ ) during the third phase has been practically always verified more or less markedly. This seems to indicate: (a) the extraordinary demand for carbohydrates by the parasite at the moment of the fructification; (b) the state of undernourishment of the



susceptible host which, when short of metabolites, can no longer satisfy the increased needs of the parasite even by increasing the rhythm of its photosynthesis. Obviously after this last effort which is advantageous exclusively to the parasite, the invaded tissue collapses and then dies.

## V. CONCLUSION

In conclusion we can say that the host is starved by pathogens (a) when the synthesis of carbohydrates and other metabolites is impaired by infections of the photosynthetic organs, (b) when respiration is too high and resynthesis of carbohydrates is inhibited, (c) when the normal equilibrium of the functions of the host is broken and the energy is wasted, (d) when the competitive action of the antimetabolites (toxins) inhibits the biosynthesis or the subsequent utilization of the metabolites, (e) when the cell permeability and then the distribution of the metabolites is injured, (f) when the transport of carbohydrates or other metabolites out from the photosynthetic organs is impaired by diseases of the phloem, or (g) when mineral nutrition is reduced by infections of the roots or xylem vessels.

## REFERENCES

- Akazawa, T., and I. Uritani. 1955a. Phytopathological chemistry of black-rotten sweet potato. 19. Inhibitory effect of bitter substances in the rotten sweet potato on *Ceratostomella fimbriata*. 20. The respiratory increase, phosphate and nitrogen metabolism in the rotten sweet potato. *J. Agr. Chem. Soc. Japan* **29**: 377-381, 381-386.
- Akazawa, T., and I. Uritani. 1955b. Respiratory increase and phosphorus and nitrogen metabolism in sweet potato infected with black rot. *Nature* **176**: 1071-1072.
- Allen, P. J. 1942. Changes in the metabolism of wheat leaves induced by infection with powdery mildew. *Am. J. Botany* **29**: 425-435.
- Allen, P. J. 1953. Toxins and tissue respiration. *Phytopathology* **43**: 221-229.
- Allen, P. J. 1954. Physiological aspects of fungus diseases of plants. *Ann. Rev. Plant Physiol.* **5**: 225-248.
- Atkinson, T. G., and M. Shaw. 1955. Occurrence of acid phosphatase in association with haustoria of powdery mildew on barley. *Nature* **175**: 993-994.
- Barton-Wright, E., and A. McBain. 1932. Studies in the physiology of the virus diseases of the potato: a comparison of the carbohydrate metabolism of normal with that of leaf-roll potatoes. *Trans. Roy. Soc. Edinburgh* **57**: 309-349.
- Benson, A. A., and M. Calvin. 1950. Carbon dioxide fixation by green plants. *Ann. Rev. Plant Physiol.* **1**: 25-42.
- Braun, A. C. 1937. Beiträge zur Frage der Toxinbildung durch *Pseudomonas tabaci* (Wo. et Fo.) Stapp. *Zentr. Bakteriell. Parasitenk. Abt. II* **97**: 177-193.
- Braun, A. C. 1950. The mechanism of action of a bacterial toxin on plant cells. *Proc. Natl. Acad. Sci. U. S.* **36**: 423-427.
- Braun, A. C. 1955. A study on the mode of action of the wildfire toxin. *Phytopathology* **45**: 659-664.

- Caldwell, R. M., H. R. Kraybill, J. T. Sullivan, and L. E. Compton. 1934. Effect of leaf rust (*P. triticina*) on yield, physical characters and composition of winter wheats. *J. Agr. Research* **48**: 1049-1071.
- Collander, R. 1957. Permeability of plant cell. *Ann. Rev. Plant Physiol.* **8**: 335-348.
- Commoner, B., and P. M. Dietz. 1952. Changes in non-protein nitrogen metabolism during tobacco mosaic biosynthesis. *J. Gen. Physiol.* **35**: 847-856.
- Daly, J. M., and R. M. Sayre. 1957. Relations between growth and respiratory metabolism in safflower infected by *Puccinia carthami*. *Phytopathology* **47**: 163-168.
- Dawson, H., and J. F. Danielli. 1952. "The Permeability of Natural Membranes," 2nd ed. Cambridge Univ. Press, London and New York. p. 365.
- Deuel, H. 1954. Über Störungen des Spurenelementhaushaltes der Pflanzen durch Welketoxine (Literaturbesprechung). *Phytopathol. Z.* **21**: 337-348.
- Dimond, A. E. 1955. Pathogenesis in the wilt diseases. *Ann. Rev. Plant Physiol.* **6**: 329-350.
- Dimond, A. E., and P. E. Waggoner. 1953. On the nature and role of vivotoxins in plant disease. *Phytopathology* **43**: 229-235.
- Dufrenoy, J. 1928a. Modification des mitochondries et des plastides dans les cellules de feuilles de haricots affectées par la mosaïque. *Compt. rend. soc. biol.* **98**: 373-374.
- Dufrenoy, J. 1928b. Observation sur les modifications pathologiques de la forme des vacuoles des cellules végétales. *Ann. Épiphyties* **14**: 227-268.
- Dufrenoy, J. 1932. Die Viruskrankheiten. *Phytopathol. Z.* **1**: 85-90.
- Farkas, G. L. 1957. Some notes on the metabolic interaction between host and parasite. *Acta. Biol. Acad. Sci. Hun.* **7**: 315-323.
- Farkas, G. L., and Z. Király. 1955. Studies on the respiration of wheat infected with stem rust and powdery mildew. *Physiol. Plantarum* **8**: 877-887.
- Forward, D. F. 1932. The influence of altered host metabolism upon modification of the infection type with *Puccinia graminis tritici* p. f. 21. *Phytopathology* **22**: 493-555.
- Fuchs, W. H., and E. Kotte. 1954. Zur Kenntnis der Resistenz von *Solanum tuberosum* gegen *Phytophthora infestans*. *Naturwissenschaften* **41**: 169-170.
- Fuchs, W. H., and R. Rohringer. 1955. Biochemische Veränderungen im Weizenblatt durch Infektion mit *P. graminis tritici*. *Naturwissenschaften* **42**: 20.
- Gassner, G. 1927. Die Frage Rostanfälligkeit als ernährungsphysiologisches Problem. *Angew. Botan.* **9**: 531-541.
- Gassner, G., and W. Straib. 1928. Untersuchungen über die Infektionsbedingungen von *P. glumarum* und *P. graminis*. *Arb. biol. Reichsanstalt. Land- u. Forst-wirtsch. Berlin-Dahlem* **16**: 609-629.
- Gäumann, E., and O. Jaag. 1947. Die physiologischen Grundlagen des parasitogenen Welkens I. *Ber. schweiz. botan. Ges.* **57**: 3-34.
- Gäumann, E., and S. Naef-Roth. 1954. Über die chelierende Wirkung einiger Welketoxine. I. *Phytopathol. Z.* **21**: 349-366.
- Gäumann, E., and S. Naef-Roth. 1955. Über die chelierende Wirkung einiger Welketoxine. II. Die Verschiebungen der Toxizität durch steigende Zusätze von Asche aus jungen Tomatensprossen. *Phytopathol. Z.* **23**: 147-160.
- Gäumann, E., and S. Naef-Roth. 1956. Über die etc. IV. Die Verschiebungen der Toxizität durch steigende Absättigung mit verschiedenen Schwermetallionen. *Phytopathol. Z.* **25**: 418-444.

- Gaumann, E., S. Naef-Roth, P. Reusser, and A. Ammann. 1952. Über den Einfluss einiger Welketoxine und Antibiotica auf die osmotischen Eigenschaften pflanzlicher Zellen. *Phytopathol. Z.* **19**: 160-220.
- Gaumann, E., S. Naef-Roth, and H. Kern. 1955. Über die etc. III. Die Verschiebungen der Toxizität durch steigende Absättigung mit Eisenionen. *Phytopathol. Z.* **24**: 373-406.
- Gottlieb, D. 1944. The mechanism of wilting caused by *Fusarium bulbigenum* var. *lycopersici*. *Phytopathology* **34**: 41-59.
- Greenshikov, A. I. 1936. The physiology of the inoculation period in rust infections. *Compt rend. acad. sci. U.R.S.S.* **2**: 245-247.
- Harris, H. A. 1940. Comparative wilt induction by *Erwinia tracheiphila* and *Phytomonas stewartii*. *Phytopathology* **30**: 625-638.
- Hassebrauk, K. 1940. Zur Frage der Wirkung Aussenfaktoren auf verschiedene Stadien von Weizenbraunrostinfektionen. *Phytopathol. Z.* **12**: 490-508.
- Hassebrauk, K. 1952. Untersuchungen über die Einwirkung von Sulfonamiden und Sulfonen auf Getreideroste. II. Weitere Unters. über die rosthemmende Wirkung. III. Unters. über den Wirkungsmechanismus von Sulfonamiden und Sulfonen. *Phytopathol. Z.* **18**: 453-460; **19**: 56-78.
- Hosson, H. H., and V. M. Cutter. 1951. The isolation and culture of *Gymnosporangium juniperi-virginianae* Schw. upon artificial media. *Proc. Natl. Acad. Sci. U. S.* **37**: 400-403.
- Humphrey, H. B., and J. Dufrenoy. 1944. Host-parasite relationship between the oat plant (*Avena* spp.) and crown rust (*P. Coronata*). *Phytopathology* **34**: 21-40.
- Kern, H. 1956. Problems of incubation in plant diseases. *Ann. Rev. Microbiol.* **10**: 351-368.
- Kokin, A. J., and C. S. Toumarinson. 1934. The physiological basis of the injuriousness of the oat rust *Puccinia coronifera*. *Bull. Plant Protect. Ser. II. Phytopathol.* **6**: 5-34.
- Kuprevicz, V. F. 1947. The physiology of the diseased plant in relation to the general questions of parasitism. *U. S. S. R. Acad. Sci., Moscow-Leningrad.* pp. 299.
- Linskens, H. F. 1955. Der Einfluss der toxischen Welke auf die Blattausscheidungen der Tomatenpflanze. *Phytopathol. Z.* **23**: 89-106.
- Mains, E. B. 1917. The relation of some rust to the physiology of their hosts. *Am. J. Botany* **4**: 179-220.
- McLean, W. 1926a. Effect of leaf-roll disease in potatoes on the composition of the tuber and "mother tuber." *J. Agr. Sci.* **16**: 318-324.
- McLean, W. 1926b. The control of leaf-roll disease in potatoes by the diagnosis of "primarily infected" tuber. *J. Agr. Sci.* **16**: 149-157.
- Miller, A., and K. Scott. 1956. Host pathogen relations in powdery mildew of barley. II. Changes in respiratory pattern. *Australian J. Biol. Sci.* **9**: 37-44.
- Montemartini, L. 1904. Note di fisiopatologia vegetale. *Atti Ist. Botan. Univ. Pavia* [II] **9**: 39-97.
- Murphy, H. C. 1935. Effect of crown rust infection on yield and water requirement of oats. *J. Agr. Research* **50**: 387-411.
- Murphy, H. C. 1936. Effect of crown rust on the composition of oats. *Phytopathology* **26**: 220-234.
- Murphy, P. A. 1923. On the cause of rolling in potato foliage; and on some further insect carriers of the leaf-roll disease. *Sci. Proc. Roy. Dublin Soc.* **17**: 163-184.

- Novikoff, V. A. 1937. Derangement of metabolism in the leaves of lucerne when infected with the rust *Uromyces striatus* Schrött. *Compt. rend. acad. sci. U.R.S.S.* **15**: 53-56.
- Ottolenghi, E., A. Shkjezi, and C. Sempio. 1953. Abito xerofitico in relazione alla resistenza. *Ann. Fac. Agrar. Univ. Perugia* **9**: 196-205.
- Pohjakallio, O. 1932. Significance of different sugars as nutrient media for some rust. *Suomen Maataloustieteellisen Seuran Julkaisu* **25**: 1-94.
- Quanjier, H. M., H. A. A. Van Der Lek, and J. O. Botjes. 1916. Nature of spreading and combating phloem-necrosis (leaf-roll) and allied diseases. *Mededel. R. Hoog. Land. Tuin. Boschlouw. Wageningen*. **10**.
- Rothstein, A. 1954. The enzymology of the cell surface. *Protoplasmatologia* [II] **E4**: 86.
- Rubin, B. A., E. P. Chetverikova, and E. W. Arzichowskaja. 1955. *Zhur. Obshchei Biol.* **16**: 106. (from Farkas, 1957).
- Sempio, C. 1938a. Primo contributo alla conoscenza dell'azione esercitata da vari fattori ambientali su alcune malattie parassitarie di piante coltivate (Ruggine del fagiolo). *Riv. Patol. vegetale* **28**: 241-351.
- Sempio, C. 1938b. Sulla maggiore sensibilità di piante infette al momento della sporificazione del parassita. *Riv. Patol. vegetale* **28**: 393-397.
- Sempio, C. 1939. Influenza della luce e dell'oscurità sui principali periodi del parasitamento. *Riv. Patol. vegetale* **29**: 1-70.
- Sempio, C. 1942a. Influenza di alcuni glucidi isomeri sullo sviluppo della ruggine del fagiolo e di altre malattie fungine. *Riv. biol. (Perugia)* **34**: 52-56.
- Sempio, C. 1942b. Terzo contributo alla conoscenza dell'azione esercitata da vari fattori ambientali su alcune malattie parassitarie di piante coltivate (*Peronospora* della lattuga). *Riv. biol. (Perugia)* **34**: 22-47.
- Sempio, C. 1942c. Respirazione, glicolisi e traspirazione nel corso del parasitamento. *Ann. Fac. Agrar. Univ. Perugia* **1**: 131-143.
- Sempio, C. 1946. Metabolisme du "complexe" Froment-Erysiphe graminis. *Monit. intern. protég. plantes inst. inter. agr. Roma* **20**: 53-69.
- Sempio, C. 1950a. Metabolic resistance to plant diseases. *Phytopathology* **40**: 799-819.
- Sempio, C. 1950b. Difesa, predisposizione e malattia intese come squilibri funzionali. *Phytopathol. Z.* **17**: 287-292.
- Shaw, M., S. A. Brown, and D. Rudd Jones. 1954. Uptake of radioactive carbon and phosphorus by parasitized leaves. *Nature* **173**: 768-769.
- Shaw, M., and D. J. Samborski. 1956a. The physiology of host parasite relations. I. The accumulation of radioactive substances at infections of facultative and obligate parasites including tobacco mosaic virus. *Can. J. Botany* **34**: 389-405.
- Shaw, M., and D. J. Samborski. 1956b. The physiology of host-parasite relations. II. The effect of *P. graminis* trit. Eriks. and Henn. on the respiration of the first leaf of resistant and susceptible species of wheat. *Can. J. Botany* **34**: 601-619.
- Shaw, M., and D. J. Samborski. 1957. The physiology of host-parasite relations. III. The pattern of respiration in rusted and mildewed cereal leaves. *Can. J. Botany* **35**: 389-407.
- Thatcher, F. S. 1939. Osmotic and permeability relations in the nutrition of fungus parasites. *Am. J. Botany* **26**: 449-458.
- Thatcher, F. S. 1942. Further studies of osmotic and permeability relations in parasitism. *Can. J. Research [C]* **20**: 283-311.



- Thatcher, F. S. 1943. Cellular changes in relation to rust resistance. *Can. J. Botany* **C21**: 151-172.
- Trelease, S. F., and H. M. Trelease. 1929. Susceptibility of wheat to mildew as influenced by carbohydrate supply. *Bull. Torrey Botan. Club* **56**: 65-92.
- Uritani, I., T. Akazawa, and M. Uritani. 1954. Increase of respiratory-rate in sweet potato tissue infected with black rot. *Nature* **174**: 1060.
- Uritani, I., T. Akazawa, and M. Uritani. 1955. Antibiotic effect on *Ceratostomella fimbriata* of ipomeamarone, an abnormal metabolite in black rot of sweet potato. *Science* **121**: 216-217.
- Vishniac, W. 1955. Biochemical aspects of photosynthesis. *Ann. Rev. Plant. Physiol.* **6**: 115-134.
- Waggoner, P. E., and A. E. Dimond. 1955. Production and role of extracellular pectic enzymes of *Fusarium oxysporum* f. *lycopersici*. *Phytopathology* **45**: 79-87.
- Walker, J. C., and M. A. Stahmann. 1955. Chemical nature of disease resistance. *Ann. Rev. Plant. Physiol.* **6**: 351-366.
- Waters, C. W. 1926. The reactions of bean rust grown on leaves in solution. *Papers Mich. Acad. Sci.* **5**: 163-177.
- Waters, C. W. 1928. The control of teliospore and unrediniospore formation by experimental method. *Phytopathology* **18**: 157-213.
- Whitehead, T. 1927. Phloem-necrosis and starch accumulation in potato leaf roll. *Rept. Brit. Assoc. York.* (from T. Whitehead, "The Potato in Health and Disease," Oliver & Boyd, Edinburgh, 1953).
- Wildman, S. G., C. C. Cheo, and J. Bonner. 1949. The protein of green leaves. III. Evidence of the formation of tobacco mosaic virus protein at the expense of a main protein component in tobacco leaf cytoplasm. *J. Biol. Chem.* **180**: 985-1001.
- Woolley, D. W., R. B. Pringle, and A. C. Braun. 1952. Isolation of the phytopathogenic toxin of *Pseudomonas tabaci*, an antagonist of methionine. *J. Biol. Chem.* **197**: 409-417.
- Woolley, D. W., G. Schaffner, and A. C. Braun. 1955. Studies on the structure of the phytopathogenic toxin of *Pseudomonas tabaci*. *J. Biol. Chem.* **215**: 485-493.
- Yarwood, C. E. 1955. Accumulation of chemicals in diseased areas of leaves. *Phytopathology* **45**: 43-48.
- Yarwood, C. E., and J. F. L. Child. 1938. Some effects of rust infection on the dry weight of host tissues. *Phytopathology* **28**: 723-733.
- Zähner, H. 1955. Über den Einfluss der Ernährung auf die Toxinempfindlichkeit von Tomatenpflanzen. *Phytopathol. Z.* **23**: 49-88.

## CHAPTER 9

# Water Is Deficient

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## I. INTRODUCTION

Life, in all probability, originated in water and in its primary state is adapted to function only in an aquatic medium, at least, at its saturation level, if not immersed in it. As the various organisms, both plants and animals, extended their habitat to life on land, they were continually subjected to the threat of desiccation and death by forces of nature, against which they retain water in themselves, but the struggle to counterbalance this threat seems to be, in a large measure, the essence of life. The title of this chapter indicates only this aspect of the life process and the problem assumes a greater significance when we are dealing with organisms put to an additional disadvantage—the "disease" which is attended by various repercussions on key metabolic functions.

Among the most important constituents of plants, water is quantitatively by far the most abundant. Protoplasm, the physical basis of life, is itself a hydrated gel, containing water, no less than 80–90% of its total weight in its active state (Levitt, 1956). It follows, therefore, that any disturbance to the normal process of life would have a marked effect on this component. The importance of water can be better appreciated if we list its various roles in the life processes of plants. In addition to its being an essential constituent of living protoplasm, it forms a continuous phase permeating the entire plant body and it takes part in various chemical reactions, particularly hydrolysis and photosynthesis. It also plays an essential role in maintaining the form and structure of herbaceous plant tissues through the maintenance of cell turgidity. The various physiological functions of protoplasm itself are controlled by the extent of its hydration. It would, thus, seem clear that any disturbance to the water economy would naturally lead to various physical and physiological consequences.

The bulk of water in plants is not in a static condition but is part of a hydrodynamic system which operates as one unit and is composed of the balancing forces at the cellular level. The water content of the various cells and tissues is in a continuous state of change. To understand fully how and why such changes in water content occur, requires consideration of the chemical and physical characteristics of various parts of the cell, of the forces which hold water in cells and of the principles which govern water movement between cells and their environment.

Water is held in the cells of plants, principally by osmotic and imbibitional forces. Osmotic forces are developed by the presence of solutes which decrease the activity or free energy of water molecules, resulting in a decrease in its diffusion pressure. Imbibition occurs because of the attraction for water of various hydrophilic colloids such as cellulose and proteins. In this case, water is held in the microcapillary lattices of complex molecules and held largely by surface forces. Thus, the free energy of water is reduced severally in plant cells and the amount by which the diffusion pressure of water is reduced is termed the diffusion pressure deficit (DPD). The DPD of a solution is equal to its osmotic pressure, but in plant cells this is greatly modified by the wall pressure (which is equal and opposite to turgor pressure) and may decrease from a value almost equal to the osmotic pressure of the cell sap (when turgor pressure is equal to zero) to zero as the turgor and wall pressures increase. The DPD of a cell would, therefore, be equal to the osmotic pressure of a solution in which it neither gains nor loses water. In other words, the water content of the cell remains unchanged as long as the DPD of its surroundings equals that of its own. Any change in either of the two

results in a movement of water from within or without the cell. Besides these osmotic forces, there is an "active" absorption of water by the cells which involves expenditure of energy and this resembles the accumulation of ions. Loss of water from the cells would, thus, involve a disruption of the equilibrium that contributes to its retention in them.

Disease manifests itself primarily in various functional disturbances, most of which, if not all, can be traced either directly or indirectly to a disturbed water balance in plants. This fact becomes evident when we study the various derangements attending on disease and trace their bearing on the water economy of the individual. But, to appreciate this, it is necessary to have a basic picture of the various means by which a plant strives to keep up its water balance and maintains its norm. Excellent reviews and treatises have appeared on this subject and the reader is referred to these (Kramer, 1955, 1956a, b, c; Meyer, 1956; Levitt, 1951, 1956; Stocking, 1956).

## II. WATER BALANCE IN NORMAL HEALTHY PLANTS

The maintenance of a favorable water balance in plants demands that the loss of water from the leaves by transpiration (guttation loss being negligible in most cases) shall not, except for very short periods, exceed the supply of water to them. If it does, the total volume of water in plants is reduced and a thirst develops, the most obvious symptom being wilting. If, therefore, thirst and its consequences, as we shall discuss later, are to be avoided a regular flow of water to the leaves is essential. Normally, plants obtain water from the soil through their root system. In its passage from the root hairs to the foliage, water encounters resistance. An adequate supply depends on overcoming this resistance, which varies in magnitude from plant to plant and, probably, from time to time within the same plant.

Absorption of water is influenced by conditions that affect the metabolism and permeability of root cells, such as low oxygen tension, high  $\text{CO}_2$  concentration and also the total soil moisture stress at the root surface. It is also accentuated by the factors of root density and root elongation which determine the distance water has to move and so influence the total amount of water available (Slatyer, 1957). The rate of transpiration determines the extent of water deficit and, hence, the amount of water needed to attain equilibrium. In most plants studied, there is a distinct lag in the rate of absorption of water as compared with the rate of transpiration during daylight hours, showing a condition of internal water deficit; but normally the rate of absorption exceeds transpiration during the later hours of the day when the internal water deficit is reduced to the minimum. The development of an internal water



deficit thus seems to be of almost daily occurrence in most plants during their growing season and the mechanism by which plants attain reduction of internal water deficit constitutes the dynamics of its water relations. Naturally, the forces directed to achieve this end should tend to decrease transpirational water loss and increase absorption of water from the substratum, conduction being usually incidental to these two factors. Conversely, forces that tend to disturb the normal water balance act on either of these two, or both, and thereby increase the internal water deficit, causing thirst.

It seems to be generally accepted that the mechanism of transpiration involves evaporation of water either through the cuticle or through the stomates. Consequent on this evaporation, a gradient is set up between the evaporating surface and the trachea and water moves from the latter to the former. This process continues only as long as the gradient is maintained, which is subject to the influence of the intervening living cells. The DPD of the evaporating surface is regulated to a large extent by the opening and closing of stomata. That stomata respond to stimuli, such as light and darkness, changes in leaf water content, temperature,  $\text{CO}_2$  content of the air, shock stimuli, changes in  $\text{H}^+$ -ion concentration and ionic effects, seems well established (Heath, 1949). The greater the DPD of the tracheal contents, the less the readiness with which water moves to the evaporating surface and the more the withdrawal of water into the intermicellar spaces of the cell wall at the evaporating surface, thus greatly increasing resistance to evaporation.

Transpiration proceeds as long as the suction force at the evaporating surface exceeds that of the leaf cells and the trachea. If the leaf cells are to remain fully turgid, their suction force must be greater than that in the tracheae on the one hand, and that at the evaporating surface, on the other. Increase in either of the latter two leads to loss of turgor in leaf cells, and wilting follows. Normally, the osmotic pressure of leaf cells is kept up at a high level by the reduction of water by transpiration, on the one hand, and by accumulation of photosynthates, on the other. Any decrease in water in leaf cells produces changes, such as hydrolysis of starch and proteins, which give rise to osmotically active substances resulting in a flow of water from the trachea, creating a tension in the xylem. As long as the DPD of the tracheal contents, due mainly to their tension, is greater than the DPD of soil water and that of leaf cells exceeds both, absorption is facilitated. The significance of a high DPD of leaf cells is at least twofold. It causes the development of a high tension in the xylem, which is necessary for absorption against the resistance of root cells; at the same time it reduces transpiration. Thus, absorption of water tends to decrease the DPD gradient between the leaf

and the tracheal fluid, whereas transpiration increases it. The tensions disappear during periods of good water supply and low transpiration; hence, existence of tension can be taken to indicate some internal water deficit, which, however, is prevented from becoming serious by the action of tension itself on absorption and transpiration (Warne, 1942).

Thus, we have here a self-regulatory mechanism for preventing the development of a serious internal water deficit. The efficiency of this mechanism is limited by the osmoregulatory changes of leaf cells. It is precisely in those plants whose cell sap has a high osmotic pressure that there is possibility of considerable tension developing when conditions favor high transpiration. This in itself is likely to facilitate the intake of water by the roots and postpone development of a serious water deficit in the leaves. It would, thus, be evident that the daily variations in osmotic pressure of plant cells, particularly in the leaf, are most significant in that they swing back the water status of the plant to a condition of balance from that of imbalance (Meyer, 1956). The cycle of variations in the osmotic quantities may be quite different under environmental conditions departing from the normal, which favors high transpiration in the presence of an adequate water supply. Seasonal variations are also known in the case of perennial plants.

### III. GENERAL SYMPTOMS AND NATURE OF WATER DEFICIENCY

The most apparent effect of water deficit is loss of form in herbaceous plant tissues—as a sequel to loss of turgor—such as wilting, flagging, and drooping of leaves and stem tips. Root hairs are also believed to wilt very commonly, although such wilting cannot be ordinarily observed (Kramer, 1950). With xerophytes and plants with rigid leaves, wilting may not be apparent, although physiologically comparable conditions exist in them. Wilting occurs when the turgor pressure is almost zero and represents a stage when the DPD is equal to the average osmotic pressure. A temporary excess of transpiration over absorption results in such changes, but normally plants regain their form as more water is made available.

Of all the major plant processes, none is more obviously affected by a deficiency of water than growth, although meristematic tissues and young cells, because of their high imbibitional forces, are able to obtain water from older tissues (Anderson and Kerr, 1943; Wilson, 1948). Water deficit interferes with cell enlargement and cell division, but promotes differentiation (Meyer, 1956). Continued water deficit eventually results in cessation of growth. A decrease in normal water supply is reflected on growth largely because of a corresponding decrease in the production of auxins (Aleksseev, 1951). This effect and other metabolic changes,

detailed below, produce plants with a stunted appearance. Rosetting of leaves takes place from shortening of internodes. Leaf area is also greatly reduced (Simonis, 1952). Reduction in root growth means decrease in available surface freely permeable to water. This is accentuated by the destruction of root hairs and premature suberization (Kramer, 1950). In general, a reduced water content of a plant, whether caused by a shortage of water in the soil, or by high transpiration rates, results in a lower shoot:root ratio on a weight basis (Meyer, 1956).

The effect of decrease in turgor on transpiration, unlike other vital functions, seems to be indirect. Transpiration is basically a passive process and as such is determined largely by the diffusion gradient from leaf to atmosphere and the rate of water supply to the roots (Slatyer, 1957). Leaf water content itself is not likely to affect transpiration unless severe wilting occurs (Gregory *et al.*, 1950); it depends on the sensitivity of the stomates to changes in turgor. A correlation between the rate of transpiration and the width of the stomatal opening is feasible only when the water content is sufficient, although stomatal behavior is, doubtless, influenced by other factors.

Reduction in leaf water content usually results in a diminished rate of photosynthesis and this is pronounced before any wilting occurs (Schneider and Childers, 1941). A loss of water of 16 to 47% or more causes a decrease of 20% in the rate of photosynthesis, but there seems to be no correlation between the amount of water lost and the intensity of photosynthesis. In plants which have recovered from wilting, the ability to carry on carbon assimilation is not restored to normalcy, but is reduced by 35 to 59% (Iljin, 1957). The influence of water deficit on photosynthesis may be direct, through a decrease in protoplasmic hydration, or indirect, through stomatal regulation (Schneider and Childers, 1941; Rabinowitch, 1945). Stomates have an important role in the absorption of  $\text{CO}_2$  from the air and in the evaporation of water. In general, water loss of even 10% induces stomatal closure. In certain species the sensitivity of the stomatal mechanism is so great, that a loss of even 3 to 5% results in their closure, e.g., *Vicia* and *Chrysanthemum* (Iljin, 1957).

Decrease in water content of cells and tissues produces important changes in their physical and chemical properties. Dehydration produces changes in viscosity and permeability of protoplasm. Moderate dehydration increases viscosity and slackens Brownian movement. A slow adaptation (hardening) is exhibited when dehydration is gradual, whereas when the process of dehydration is drastic, complete gelation occurs and the protoplasm becomes rigid and brittle, in which case the chances of recovery are remote (Levitt, 1956). Northen (1943) and Stocker

(1948) believed that dehydration causes dissociation of the protoplasm, resulting in the physical changes observed, followed by activation of certain enzymes and increased respiration. In general, hydrolytic processes are increased, e.g., hydrolysis of starch and proteins (Kramer, 1956a) and synthetic processes are hindered, e.g., protein formation from amino acids (Petrie and Wood, 1938).

A relatively high water content of the leaf tissues favors accumulation of starch at the expense of sugars in many species, while a reduction in water content favors the transformation of starch to sugars (Ahrns, 1924; Spoehr and Milner, 1939) or polysaccharides (Spoehr, 1919). An increased supply of available nitrogen stimulates the utilization of carbohydrates, and if in addition sufficient moisture is available, growth and formation of new organs are accelerated. On the other hand, if sufficient moisture is not available, growth is interrupted and polysaccharides tend to accumulate. The breakdown of carbohydrates in leaves may be accompanied by their deposition in roots. This happens to be the case in a majority of plants (Iljin, 1957). However, when wilting is slight, the changes detailed above are not noticed, until further desiccation stimulates these processes. It is, therefore, logical to presume that fluctuations of considerable magnitude in the water content of plants, due to their "inefficient" hydrodynamic system, result in changes which increase the proneness of the host to attacks by pathogenic, root-infecting organisms.

#### *A. Physiological Wilting*

In general, diminution of water content affects the leaf cells most, as compared with other parts of plants and this results in their partial or complete loss of turgor. Visible manifestations of wilting are frequent also in young succulent stem tips, floral parts, or even fruits and root hairs. The term "incipient wilting" is applied when the loss of turgor is not great enough to result in visible drooping. This condition is found in most terrestrial plants on bright and warm days. If the suction force of the evaporating surface is greater than that of the tracheal contents, and both are greater than that of the living cells, water may pass through the leaf cells without maintaining the latter in full turgidity. This seems to be borne out by the fact that even in a wilted leaf water continues to be transpired. The development of a considerable degree of tension in the tracheae would also cause a flow of water from the living leaf cells to the tracheae. Normally, tension in the xylem does not exist for a long time when there is sufficient water supply and time for recovery. If, however, this continues owing to a prolonged shortage of water supply, the leaves pass on to a state of permanent wilting through transient wilting. Both incipient wilting and transient wilting differ from per-



manent wilting in that the latter results not from a transitory excess of transpiration over water absorption, but from a deficiency of water in the soil. Plants do not recover from permanent wilting unless the water content of the soil in which they are rooted increases; they do not regain their turgor when placed in a saturated atmosphere. Permanent wilting marks a stage beyond which water is not available for normal plant functions. It usually arises when the soil water has fallen to such a low level that the plant cannot extract it. In other words, the total soil moisture stress increases. This stress represents the DPD of soil water and is equivalent to the combined effect of the soil moisture tension and the osmotic concentration of the soil solution (Richards and Wadleigh, 1952). As the soil water is depleted by absorption by the roots, an increase in the solute concentration at the root surface occurs. This is because the rate of solute absorption and the rate of water absorption are controlled by different factors. This causes a slackening in the rate of absorption, if the soil water is not replenished and wilting is hastened (Slatyer, 1957). In a soil slowly drying up, temporary wilting slowly grades over to permanent wilting. The nocturnal recovery of the plant from temporary wilting is achieved less and less completely until even the slightest recovery fails to take place. During permanent wilting the stress in the hydrodynamic system gradually becomes intensified; even if the stomates are closed, as they usually are in permanently wilted plants, cuticular transpiration continues, gradually reducing the total volume of water within the plant. Prolongation of this state for more than a few days results in the death of root hairs and, thereafter, recovery of the plant to the normal condition is slow (Kramer, 1950). It also results in development of high tensions in the cells and these, in turn, subject protoplasm and cell walls to a centripetally directed pull which may lead to death of cells and ultimate wilting.

Physiological wilting of plants may also result from conditions other than soil moisture deficiency. Roots may be unable to absorb water from soil because of factors that interfere with absorption. Absorption of water by the roots is both active and passive. The former involves an expenditure of energy derived from respiration and resembles the phenomenon of salt accumulation. The latter is controlled by the osmotic gradient that exists between the sap in the xylem and soil water. Any interference with either, or both, results in a physiologic drought. These two processes are, however, interrelated and controlled by the metabolic state of root cells, since salt uptake causes osmotic absorption of water (Lundegårdh, 1946). Bonner *et al.* (1953) proposed that water intake and respiration are linked by transfer of energy through adenosine triphosphate (ATP), perhaps in a mechanism wherein auxin molecules

function as a part of the transport system. Although the nature of the relation between respiration and water intake is uncertain, inhibitors of the former have been found to reduce or prevent water absorption (Van Overbeek, 1942; Kelly, 1947; Rosene, 1947; Hackett and Thimann, 1952). Absorption is also impeded by flooding of the soil. This results in "flooding injury" which manifests itself in yellowing and wilting of leaves. These symptoms are attributed to desiccation caused by a decreased absorption. The picture is complicated further by the injury and death of root cells due to poor aeration. This, however, does not seem to explain wilting and death of shoots adequately, since plants can live for some days after their root systems are killed, if the soil is kept saturated with water (Kramer, 1933). It, therefore, appears probable that the various symptoms produced by flooding have several causes, in addition to interference with absorption, such as inhibition of root growth, root development and elongation, translocation of toxic substances either released from dying cells (e.g., ethylene) or produced in the soil (Kramer, 1951), and nonselective absorption of minerals as a sequel to the death of the root system. An imbalance of minerals is known to cause injury to leaves, such as mesophyll collapse noticed in citrus leaves (Sokoloff *et al.*, 1943).

### B. Pathological Wilting

Pathological wilting, as the term denotes, is caused by pathogenic agencies. It occurs in diseases variously described as damping-off, die-back, foot rot, take-all, and wilt. Damping-off is caused by primitive parasites which attack the seedlings of many plants and bring about their death by extensive rotting of the root and collar region (e.g., species of *Fusarium*, *Pythium*, and *Rhizoctonia*). Foot rot and take-all represent instances wherein the roots and crown regions are damaged and the plants exhibit characteristic symptoms of water deficiency before they die. In the case of foot rot of paddy (*Gibberella fujikuroi*), however, the presence of characteristic metabolites of the causal organism—fusaric acid and gibberellic acid, the former a wilt toxin and the latter having the property of growth substances—has been recently demonstrated (Subba-Rao, 1957a, b). The final symptoms of the disease, in this case, would naturally arise not only from the interference with water uptake consequent to the damage to subterranean portions of the plant, but also as a result of the presence of these metabolites. Fusaric acid in low concentrations increases permeability of protoplasts to water and reduces it at higher concentrations. Gibberellic acid, on the other hand, inhibits root growth among other things and this would aggravate root dysfunction. The syndrome of foot rot of paddy thus appears to be the product

of an intricate interplay of diverse factors (Gäumann, 1957). In the case of take-all no such evidence has been brought forward so far and the disease appears to arise out of the dysfunction of root system resulting from injury by the causal organism, *Ophiobolus graminis* (Ludbrook, 1942). Dieback of plants and branches is caused by definite interference with water supply, either by tissue disintegration or by inducing gum formation in the xylem region.

In vascular wilt diseases, however, there are to be seen both a local effect which leads to the necrosis of the directly affected tissues and a general systemic effect leading to the death of the entire plant. Wilting may occur in most instances, although it is not evident in such cases as cabbage yellows caused by *Fusarium conglomerans*. Most of the vascular wilts have many features in common, such as vascular discoloration, epinasty, yellowing, and vein-clearing, and this suggests a common basis for their origin. There is a general derangement in the water balance observable in the infected plants, but the exact manner in which it is brought about still defies a precise answer.

Damage to the absorptive organs has sometimes been suggested as a principal factor in the disease (Orton, 1902), but this does not seem to be apparent. In most cases the extent of root damage is too meager to account for such an acute water shortage and often wilting occurs before any visible damage to the root is noticeable. Injury to roots, causing death of root cells, will not interfere with the supply of water to the shoot, but a functional disturbance may lead to indiscriminate passage of toxic compounds and minerals through them and thereby cause damage to the shoot. Recent work on *Fusarium* wilt of cotton seems to provide evidence for such an occurrence (Gnanam, 1956; Sadasivan, 1957).

There is considerable evidence that dysfunction of conductive elements causes an acute water shortage. This appears to be brought about by various physical and chemical causes, such as, the presence in the tubes of wefts of hyphae and masses of organisms (as in bacterial wilts) or gums, tyloses, gels, and gas pockets, all arising out of the chemical activities of the parasite during its interactions with the host. These may interfere with water flow through the xylem, either by obstructing the free passage or by increasing the viscosity of the tracheal fluid. However, in view of the present state of knowledge of the mechanism of sap flow through the xylem, these barriers seem to have only a limited significance (Dimond, 1955). In addition to a general water shortage, there are symptoms such as vein-clearing, epinasty, and necrosis in the leaves, which indicate the operation of a translocated systemic factor. The course of transpiration in diseased plants, and the exudations of minerals and

amino acids from leaves, as shown by Linskens (1955), indicate that the leaf cells have lost their normal functions of osmoregulation. The role of pectic enzymes, initiating a chain of reactions leading to the release of phenols in the transpirational stream, is sometimes suggested to explain this derangement (Davis *et al.*, 1953; Dimond, 1955). Poisoning of leaf cells by metabolites of the causal agent carried along the sap stream is also suggested to account for this condition. The mechanism by which the toxin(s) brings about such changes appears to be primarily due to the destruction of the osmotic prerequisites for turgor (Gäumann and Jaag, 1947), causing a release of cellular components into the outer medium, which appears to be the basis of pathological wilting in plants (Gäumann, 1951). This results in the loss of water-retaining capacity of the leaf cells and an efflux of water into the transpirational stream causing an increase in water loss. Mere shortage of water will not produce such effects as claimed by many; on the other hand, shortage of water may reduce the leaf area, but often increases water content of leaves possibly by changes in the protoplasmic structure which enable it to bind more water (Simonis, 1952). Such a condition does not seem to exist in most of the wilt diseases. The loss of water-retaining capacity of the leaf cells, however, seems to be reversible in many cases (Dimond, 1955), and Gottlieb (1944) envisages the continued action of a slow acting poison.

All this, regarding the role of enzymes and toxins, as well as other toxic substances in the production of disease syndromes, has remained largely speculative until very recently, having been mostly based on *in vitro* studies. The ability of the pathogens concerned to produce these substances *in vitro* could not always be correlated with the degree of their pathogenicity under natural conditions. The controversy that naturally arose out of this incompatibility led to the development of the new concept of "vivotoxin" (Dimond and Waggoner, 1953a), i.e., a toxin operative *in vivo*. Although the term has been coined to define toxin action, the idea can be extended to bring within its scope the many other products ascribed a role in pathogenesis. A rigorous application of Koch's postulates, as applied for the establishment of parasitism by microorganisms would, however, seem impossible when we consider the conditions in which these factors act *in vivo*. The secretion and activity of enzymes are limited by the presence of adequate substrates and optimal conditions for their activity. Vivotoxins, on the other hand, are most probably continuously secreted and continuously inactivated or destroyed by host reactions. Hence, the establishment of their complicity in initiating certain aspects of wilt syndrome would be difficult by *in vitro* studies.



Nevertheless, recent studies by many workers have been instrumental in demonstrating the presence of pectic enzymes and metabolites of the pathogen, such as fusaric acid *in vivo* in the infected plants, together with the presence of the products of host-parasite interaction, such as ethylene and phenols (Davis *et al.*, 1953; Dimond and Waggoner, 1953a, c; Gothoskar *et al.*, 1953, 1955; Kern and Sanwal, 1954; Lakshminarayanan and Subramanian, 1955; Waggoner and Dimond, 1955, 1956; Kalyanasundaram and Venkata Ram, 1956; Kern and Kluepfel, 1956; Subramanian, 1956; Husain and Kelman, 1957; Lakshminarayanan, 1957). A recognition of their presence *in vivo* in the diseased plants strongly suggests the possibility of their taking part in the initiation of the array of symptoms observed during pathogenesis in wilt diseases. The pathogenesis of toxins is discussed in more detail in Chapter 9 of Volume II.

The similar symptoms of many wilt diseases suggest a common biochemical basis for their origin. Still, it is difficult to suggest a single comprehensive mechanism of disease initiation for all, in view of the fact that the relative importance and the presence of these factors would depend on the different host-parasite complexes.

#### IV. "WATER IMBALANCE" IN DISEASED PLANTS

A change in the water content may, perhaps, be one of the earliest reactions of living cells to any disturbance. Most diseases are characterized by an initial increase in transpiration rate which, in itself, is sufficient to upset the water economy of a plant. There are, however, instances where a reduction of transpiration rate has been observed, e.g., bacterial wilt of cucumbers (Yu, 1933). The increase in transpiration may be brought about by various causes—physical or chemical. Changes in permeability of leaf cells are almost universally observed. The protective influences of the cuticle may sometimes be subverted by ruptures caused by fructifications of pathogens (as in rusts). The regulating influence of stomates may be altered, notably by variations in the starch-sugar equilibrium. The damage to leaf cells by diffusible toxic substances, released from the focus of infection, would contribute to the changes in leaf behavior.

The net effect of infection, in most of the diseases studied, is a gradual decline in transpiration rate, although there may be an initial increase in water loss. This decline, in many instances, points to an inability of the infected plants to obtain enough water to maintain their turgidity and would appear to be occasioned by an impediment to absorption and conduction, resulting in an increased internal water deficit, aggravated by loss of control over transpiration.

A disturbance in auxin balance due to disease is reflected in the

growth pattern of plants. The affected plants are unthrifty and exhibit poor growth. An increase in absorption may be expected to compensate for this imbalance, but this does not take place since cessation of root growth and severe root damage observed in many diseases delimit this possibility.

The conduction of water through the xylem may be variously impeded: (1) by the presence of the pathogen, (2) by the chemical activities of the pathogen resulting in formation of gums and gels, (3) by stimulation of host reactions leading to hyperplastic development of xylem parenchyma and tylose formation.

The gross metabolic changes coupled with mechanical barriers, produced in the host by host-parasite interaction, exert a marked influence on the water content and turnover of cells, and produce changes in the internal distribution of water in tissues and organs. The effect is initially localized, but becomes systemic in course of time. All these changes are reflected in the variety of symptoms manifested in the diseased condition, ranging from stunting and rosetting to wilting and drying up. The conditions leading to these are considered in detail in the following sections.

#### *A. Absorption—the Dysfunction of the Root*

Reduction in the efficiency of roots as absorbing organs is noticed in many diseases, such as root rots (Simmonds, 1939), viroses (Stubbs, 1947), and rusts (Johnston and Miller, 1934; Bever, 1937). This results from root damage which may be produced in various ways.

##### *1. Root Growth Is Affected*

It is generally assumed that root elongation and production of root hairs significantly increase water uptake by increasing the absorbing surface in contact with the soil. This is not necessarily true, since absorption is not always limited by the root surface in contact with the external medium, particularly if the water supply to the soil is adequate, but by internal factors, such as permeability of root tissues, the metabolic state of root cells, the capacity of the xylem to conduct water, and the gradient of the diffusion pressure deficit between the soil solution and xylem sap (Kramer, 1956c). In the case of diseased plants, suffering from a serious internal water deficit, however, the root density and extent are of considerable importance. A study of many examples shows that, wherever fresh production and growth of roots are initiated, the plants have a better chance of surviving disease; for instance, Mostafa (1954) demonstrated that fungal filtrates often stimulated rooting in disease-resistant varieties and he claimed this to be the mechanism of resistance in those plants. Reduction in growth is caused primarily by decrease in the water status

of plants. This is because of a consequent increase in osmotic value, beyond the optimal, which reduces the intensity of vital activities.

Loss of turgor, due to decrease in water content, in the shoots is presumably accompanied by loss of turgor in the roots, resulting in injury to or destruction of root hairs and reduction in or cessation of root elongation. Suberization of the epidermis and tissue differentiation are rapid with a corresponding decrease in root elongation and consequent reduction in the proportion of root surface freely permeable to water (Kramer, 1950).

Soil conditions affecting root growth include physicochemical properties of the soil, namely, texture, aeration, availability of moisture, and the total soil moisture stress. In addition to these, the presence of soil microflora, especially in the region surrounding the root—the rhizosphere—markedly influences root development. Many of these are known to synthesize and release into the soil medium metabolites which influence root growth and character (Norman, 1955; Brian, 1957). The capacity of plant roots to respond to externally applied (present?) growth factors varies in different species (Kato, 1957). Generally, only inhibitory effects (on root elongation) have been reported, since the auxin content of intact roots is normally above optimum (Åberg, 1957). In the “bakanae” disease of rice, an interesting situation presents itself. The pathogen *Gibberella fujikuroi* secretes gibberellins which have growth stimulatory properties; but this effect is seen only in the shoots while root growth is inhibited. The other apparent effects of the microbial mantle, such as lowering the oxygen tension and increasing the  $\text{CO}_2$  concentration, may also affect growth and extent of the root system. Farr (1924) presented evidence for the inhibition of root hair production in the presence of the pathogen *Fusarium lycopersici* in susceptible tomato varieties, whereas the resistant varieties behaved normally. Generally, root hair production is inhibited by lack of oxygen (Snow, 1905). It is logical to expect a lowered oxygen concentration around the roots of plants supporting quantitatively higher rhizosphere microfloras. It is well established in the case of many diseases that susceptible varieties exert a greater “rhizosphere effect” than their resistant counterparts.

## 2. Roots Are Injured

An extensive destruction of the root system precedes the appearance of above ground symptoms in the case of root rot, foot rot, and other diseases caused by primitive root-infecting fungi. In many wilt diseases, however, extensive killing of the root system is postponed until after the host succumbs to the disease. Death of the root system seems to be

less serious than the functional alteration in a living root system. In the former case, the osmotic barrier of the root is destroyed and water flows into the shoot by mass flow, whereas in the latter, a metabolic depression of the root cells causes a decreased absorption. Damage to tips and younger portions, rather than older parts of the roots, is more detrimental to the plant. Effects of root infections in several cases are comparable to mechanical root injuries in many respects (cf. Ludbrook, 1942; Simmonds, 1939). Crandall *et al.* (1945) report that the symptoms of a loss of color of the foliage, followed by wilting in the broad leaved species and dieback in conifers, do not appear until the roots are almost completely rotted. Heavy infection by certain cereal rusts has resulted in a rapid and severe deterioration of the root system characterized by discoloration, decrease in the number of fibrous roots, and marked loss in weight (Johnston and Miller, 1934; Murphy, 1935).

### 3. *Permeability of Root Cells Is Altered*

Permeability of root cells may be affected in many ways by disease. Changes in the metabolic status of cells affect the active uptake of water by them. The changes in the osmotic gradient, which again is controlled by metabolic changes to some extent as Lundegårdh (1946) points out, may alter the osmotic movement of water through the roots.

As we have seen earlier, a water deficit occurs during disease and this results in a reduction in the hydration of the protoplasm, which leads on to a depressed metabolic activity. The metabolic activity of root cells is also subject to the action of external factors, such as microbial activities and their consequences in the rhizosphere, the amount of oxygen and nature of salts present in the surrounding medium, etc. The possibility that water absorption, like salt accumulation, is not merely an equilibrium process but may involve an internal secretion peculiar to cells which are still able to grow, is suggested by the work of Bennet-Clark *et al.* (1936). Internal water deficit and the presence of growth depressants (antibiotics and growth factors) in the environment retard growth of root cells and inhibit synthetic processes; the latter may seriously interfere with permeability. Root cells subjected to the action of antibiotics, such as polymyxin, liberate their cell contents into the medium and it is quite reasonable to expect that metabolites which are produced in soils around the roots affect the root cells in a similar way (Norman, 1955). Moreover, any injury to root cells results in a leakage of cell contents into the medium. As a rule, healthy cells do not lose their contents, while injured cells do (Helder, 1956). It may be reasonable to presume that substances rich in nutrient value are exuded from



diseased roots, thus providing a good substrate for increased microbial activity. This, in turn, could be expected to change the  $O_2$  and  $CO_2$  concentrations, bringing about further changes in root function.

Where root cells are killed, they lose their selective permeability. Thus, there could be an indiscriminate entry of toxic substances—of plant and microbial origin—and minerals (Kramer, 1951). Many symptoms observed in aerial parts of diseased plants may be traced to such a nonselective passage of substances through the damaged root system. How toxic materials affect living cells has already been described. Plants affected by diseases such as foot rot and root rot exhibit signs of mineral deficiency (Jenkins, 1948). A mineral imbalance causes injury to leaves, such as mesophyll collapse observed in citrus (Sokoloff *et al.*, 1943). A pronounced ionic derangement has been noticed in the *Fusarium* wilt of cotton (Sadasivan and Kalyanasundaram, 1956; Sadasivan and Saraswathi-Devi, 1957). All these data strongly point to the fact that there is a dysfunction of the root system resulting in a loss in selective permeability and absorption.

#### B. Conduction—the Dysfunction of Conductive Elements

The problem of separating the factors affecting absorption from those affecting conduction is a difficult one since both the processes are interdependent. Fluometric studies by many workers (Melhus *et al.*, 1924; Ludwig, 1952; Dimond and Waggoner, 1953b; Beckman *et al.*, 1953) reveal that the rate of flow of water in stems of diseased plants infected with vascular parasites is reduced considerably. Many authors claim that wilting of the affected plants is due to an impairment in conduction. Powers (1954) attributes the cause for wilting in tobacco affected by "black-shank" as due to the impairment of water movement through the lesions produced in the stem by *Phytophthora parasitica* var. *nicotianae*. Keyworth (1953), working on the *Verticillium* wilt of the hop, found that the severity of leaf symptoms is determined by stem invasion. The mean rate of flow is less than half as great in stem tissues of soybean plants invaded by the fungus *Cephalosporium gregatum* as in healthy tissue of comparable stem size (McAlister and Chamberlain, 1951). These workers also find an inverse relationship between the degree of browning in the vascular system and the rate of water flow. The occlusion of conductive elements in the petioles of leaves seems to be of greater significance than that in the stems inasmuch as there is little scope for circumventing this obstruction, due to lack of an alternate path for conduction and absence of secondary thickening.

The various materials that are known to contribute to the dysfunction of conductive elements include the physical presence of the organism

and/or products of its chemical activity in the conductive elements. The reaction of the host tissues to the presence of the organisms in its interior also contributes a share in the production of occluding materials. A consideration of the nature, origin, and effect of these on water flow is presented in some detail below.

### 1. *Vessels Are Choked*

a. *Organisms*. A mechanical blockage of vessels has sometimes been attributed to wefts of hyphae growing freely into the lumen of the vessels and, in the case of bacterial diseases, to slimy colonies of the pathogens. Grieve (1941) demonstrated in tomatoes and potatoes, infected with *Bacterium solanacearum*, that the progress of absorption in relation to invasion was closely similar to that of transpiration under the same conditions. Where the parasite was inoculated at the stem apex, no reduction in absorption took place before the bacteria had "overrun" and "blocked" several root vessels after growing downward through the stem. The distribution of the organisms in the vascular tracts was usually found to be vertical rather than lateral and, hence, the chances of the vessels' getting blocked by the lateral spread of the pathogen are negligible and those vessels that are not infected initially remain comparatively free of mycelium. Although the presence of the organism in the lumen of vessels is noticed by many workers, the inadequacy of such an obstruction to cause the marked changes observed seems to have been realized by them. This phenomenon, however, merits consideration as a contributory factor to the reduction in water flow.

b. *Enzymes*. The production of pectic enzymes by a number of vascular wilt pathogens and their role in pathogenesis have been investigated in detail in recent years (Scheffer and Walker, 1953; Gothoskar *et al.*, 1953, 1955; Waggoner and Dimond, 1955; Kamal and Wood, 1956; Subramanian, 1956; Lakshminarayanan, 1957). These enzymes act on the middle lamellae exposed at the pit region and liberate pectic acids and other products of partial hydrolysis of pectin, which form gels combining with metals. If the hydrolysis proceeds further, gum formation takes place. The presence of these enzymes *in vivo* has been demonstrated in some cases; for instance, in the *Fusarium* wilt of tomato (Waggoner and Dimond, 1955) and cotton (Lakshminarayanan, 1957). Both pectolytic and cellulolytic enzymes have been noticed in the bacterial wilt of tomato caused by *Pseudomonas solanacearum* (Husain and Kelman, 1957). The fact that these enzymes are produced *in vivo* suggests their importance in tissue breakdown commonly observed in these diseases. The conditions under which they act in different cases, however, seem to vary and this determines the extent of tissue breakdown. The presence

of metallic ions, particularly heavy metals (Subramanian, 1956) and alkaline earth salts (Lineweaver and Ballou, 1945), influences their activity *in vitro*. The production and activity of these enzymes also vary with the composition of the medium in which they are built up, such as the nature of the carbon source (Waggoner and Dimond, 1955) and on the chemical nature of the substrate on which they act—the degree of esterification in the case of pectin (Lineweaver and Jansen, 1951). Gäumann and Böhni (1947a, b), studying the effect of nutrient solution on the production of pectinase and pectase by *Botrytis cinerea*, showed the former to be developed independent of the chemical composition of the medium, whereas the latter, which splits the methyl alcohol in pectin, is largely adaptive, being produced in quantity in the presence of pectin, but only in traces without it. The substrate for pectinase is provided by the action of pectase which de-esterifies the pectin. The hydrolysis of pectic acid by pectinase is essentially zero until 45 to 50% of the bonds are hydrolyzed. This shows the synergistic action of both these enzymes on the breakdown of pectin. This is especially significant when we realize that pectase is largely adaptive, depending on the presence of pectin in the substratum.

The action of the pectic enzymes on the middle lamellae of the vascular elements is to macerate the tissue and this results in the development of vascular plugs (Pierson *et al.*, 1955). In tomato cuttings treated with commercial pectic enzyme preparations, this effect is seen clearly. The xylem walls appear to be thinned out and the plugs formed by the enzyme action are stained by ruthenium red revealing their pectic origin. A characteristic vascular discoloration has been observed in many wilt diseases and this has been attributed to the action of parasitic enzymes such as pectin methyl esterase (Winstead and Walker, 1954) and to the oxidation of phenols resulting in melanoid pigments (Davis *et al.*, 1953; Waggoner and Dimond, 1955, 1956). Phenols are liberated from phenolic glycosides on hydrolysis by  $\beta$ -glucosidases or by shunting off the phenols from lignin formation. The lignin content of cells of infected plants was shown to be lower, thus providing evidence for this possibility (Davis and Dimond, 1954). The polymerization of phenols appears to take place in the living cells of the xylem parenchyma. The substrate for fungal  $\beta$ -glucosidase is contained in the cells of xylem parenchyma and is made available only after a certain amount of tissue maceration by the action of pectic enzymes. Thus, the action of these pectic enzymes starts a chain of reactions in the xylem resulting in plugging and discoloration. Gäumann *et al.* (1953) isolated a fraction from the fungal filtrates of *Fusarium lycopersici* responsible for vascular discoloration, the nature of which seems to be that of an enzymatic

protein. The exact manner in which this brings about vascular discoloration is not known; perhaps it is one of these enzymes that takes part in the above mentioned sequence of actions. Chamberlain and McAlister (1954) report that the rate of water flow in diseased soybean stem affected by "brown stem rot" is inversely proportional to the degree of browning in the vascular system indicating the action of these enzymes, bringing about a reduction in water flow.

c. *Gums and Gels*. Considerable evidence has been adduced to show that pectic degradation resulting in the release of gel and gum forming substances takes place in the conductive elements and causes considerable obstruction to the efficient translocation of water to the leaves. Dark colored gums have been found in oak wilt (Struckmeyer *et al.*, 1954) and these may also contribute to vascular discoloration. The presence of gum in other cases of wilts is not detectable in the early stages, but appears only after the onset of wilting (Dimond, 1955); its formation is preceded by the appearance of homogeneous granular and gray material which is not preserved in fixed and stained sections, but is detectable only in living material. This happens because the dehydration in the process and the hydrophilic nature of the material do not permit its preservation. It is likely that the dehydrated granular mass lies adpressed to the xylem wall.

d. *Polysaccharides*. Polysaccharides and other large molecules are known to cause wilting in tomato (Hodgson *et al.*, 1949; Gäumann, 1951; Scheffer and Walker, 1953). These have been recognized as metabolic products of fungal and bacterial growth (Hodgson *et al.*, 1947; Dimond *et al.*, 1949; Dimond and Waggoner, 1953a). It is very probable that the large polysaccharide molecules are liberated in the xylem, where these organisms are growing, in sufficient amounts to cause wilting. These substances are known to cause a mechanical blockage of vascular bundles and intermicellar spaces, thus initiating a physical wilting. If pathological wilting in plants were caused by such a mechanical blockage of vascular bundles, they should behave as plants exposed to physiological wilting from shortage of water. But this does not appear to be the case in the naturally infected plants. Moreover, if such occlusions of the intermicellar spaces of the xylem were to occur, the laminar flow of vascular stream should be greatly affected and the reversibility of the wilting action and regaining of turgidity by organs, as reported by many workers (Hursh, 1928; Dimond, 1955), will not occur.

e. *Hyperplasia*. A different type of vascular obstruction has been observed in the mosaic-infected tomato stems (Gardner, 1925). In response to necrosis caused by the virus, the host cells adjacent to these necrotic spots manifest a hyperplastic development resulting in an inva-



sion of the xylem tissue and an inwardly directed pressure. Tracheal tubes which happen to lie in the path of the hyperplastic cells are crushed and their lumen completely obliterated. In cases where a considerable proportion of the circumference of the xylem cylinder is invaded by the hyperplastic growth, it is readily conceivable that the water supply to the top might be cut off. In stems of plants infected by the crown gall organism, *Bacterium tumefaciens*, such hyperplasia is known to occur and these stems conduct considerably less water than normal (Melhus *et al.*, 1924).

f. *Tyloses*. Occlusion of vessels by formation of tyloses resulting in impaired water flow has been noticed in many vascular diseases coincident with the appearance of wilt (Sleeth, 1933; Beckman *et al.*, 1953; Struckmeyer *et al.*, 1954). The formation of tyloses is attributed to various causes such as the presence of the parasite, or a reaction to the toxic substance produced by the parasite or to a chronic water shortage (Sarmah, 1956). Struckmeyer *et al.* (1954) found the formation of tyloses preceding the development of wilt symptoms and claim that this is the cause and not the effect of an impairment in conduction. Abundant tyloses were observed in melon plants infected with *Fusarium niveum*, their occurrence being apparently correlated with the presence, quantity, and proximity of the fungus (Sleeth, 1933). Powers (1954) also suggests that tyloses and gums are the main causes of obstruction of water movement in "black shank" of tobacco, rather than a result of previous rupture of the water columns. He indicates that the development of tyloses and gums in the vessels of diseased stems is induced primarily by the toxic effects of the decomposition products of the invaded cells which are not carried very far from the infection court. The formation of tyloses seems to be in no way restricted to a case of infection, since they are found in the heartwood of even healthy trees when conduction ceases. In this case the formation of these outgrowths into the xylem seems to be due to the exposure of the inner walls to air columns but injury also stimulates their production. Gum formation is sometimes attributed to the stress in the water column in the conducting vessels (Klotz, 1948) and it seems to be the normal defensive reaction of the host to mechanical, climatic, toxic, or parasitic stimuli (Bertelli, 1948). In cases where tyloses are observed, rarely are they enough to cause complete obstruction and, therefore, these cannot be considered as wholly responsible for wilting and necrosis arising out of an acute water shortage.

## 2. Viscosity Changes

The release of gums in the vascular sap may cause a change in the viscosity of the tracheal fluid. In some bacterial wilts the viscosity of the

tracheal fluid in the infected plants appears to be higher and Ludwig (1952) demonstrated an inverse relation between viscosity and the rate of sap flow. However, Dimond and Waggoner claim that the xylem sap in infected tomato plant does not show considerable change in viscosity compared to healthy ones and, therefore, this factor does not appear to contribute to the reduction in water flow in the case of *Fusarium* wilt of tomato (Dimond and Waggoner, 1953b; Waggoner and Dimond, 1954).

### 3. *Gas Emboli*

The formation of gas pockets in water columns of the infected xylem has sometimes been suggested as the cause of a water shortage at the tops of plants (Tochinai, 1926). This theory was advanced on the basis of the observations that the flax wilt organism, *Fusarium lini*, produces considerable amounts of CO<sub>2</sub> in cultures *in vitro*. That gas emboli appear in normal plants has not been completely overruled yet and we do not know how far this phenomenon would result in the breaking up of the cohesive forces in the water columns. If, however, the bubbles do not exceed the critical size, they are usually dissolved and the cohesion of the water column is maintained. Scholander *et al.* (1955) investigated the effects of gas emboli on water conduction and found that, in spite of the presence of large volumes of gas in vine stems, rates of uptake diminish only slightly or not at all when there is an adequate supply of water. They suggest that much of the sap flow is confined to the "finer structures between the vessels." More recent studies have indicated that any air breaks in the vessels and tracheids are confined to the units in which they occur and the transpiration stream simply moves around these obstacles in the intact units (Dixon, 1914; Scholander *et al.*, 1957).

### 4. *Some Considerations on "Reduction in Water Flow"*

A consideration of the mechanism by which water is transported to the leaves through the conductive elements becomes imperative in order to assess the relative importance of the various barriers to the free flow of vascular stream. Among the theories advanced, the cohesion theory of Dixon and Jolly (1895) seems to be fairly satisfactory. However, it does not explain the phenomenon entirely. Many other theories have been advanced from time to time, notable among them being the vitalistic theories first suggested by Bose (1923) envisaging a role of pumping action in the upward transport of water by the living cortical cells. Although this was severely criticized by a number of investigators (Benedict, 1927; MacDougal *et al.*, 1929; Smith *et al.*, 1931), the vitalistic theories seem to warrant some consideration. Peirce (1934, 1936) dis-

cussed the complexities of the phenomenon and concluded that it is not purely physical in nature. He observes that water is moved through the plant by physical means; however, when the living cells surrounding the vascular tissues are killed by heat, cold, or poisons, the conducting system is rendered functionless. The function of the living cells seems to consist in "maintaining a continuous but many phased water mass in the capillary body of the plant, conditioning but not compelling the ascent of sap" (Greenidge, 1957). Handley (1939), from his experiments on the effects of low temperature on the sap ascent, believes that a chain of living cells continuous from roots to leaves is involved in the ascent of sap. Many other theories have also been proposed but they are largely based on one of these described above, i.e., physical or involving the participation of living cells. Lundegårdh (1954) suggests, as an alternative to the cohesion hypothesis, that the forces involved in this phenomenon are the complex of capillary suction in the medium sized tracheids, electrocapillarity and other surface phenomena, while larger tracheids and vessels are air filled and play no role in conduction. The activity of living cells seems to be involved in the movement of water in trees. In contrast to the cohesion theory, Lundegårdh considers that only a small proportion of the total water in the xylem is mobile and the transpiration stream is confined to this relatively free fraction. It seems that "capillary forces in cooperation with the adsorptive qualities of the wall substance and the osmotic imbibition of the living tissues in the stem are so successfully contributing to the maintenance of a continuous sheath of water that an ascending sap stream can be maintained by a real suction pressure originated in the transpiring leaves of moderate height perhaps even less than one atmosphere" (Greenidge, 1957).

Viewed in the light of the present state of knowledge on the mechanism of water transport in plants, the relative importance of the various barriers considered above becomes obvious. According to the cohesion theory and that advanced by Lundegårdh involving surface forces, the state of the vessel walls assumes a great importance. By the action of the enzymes of the pathogen the composition of the vessel walls is altered, with the release of hydrophilic substances having different surface properties. The release of large molecules occluding capillaries of the intercellular and intermicellar spaces would seriously interfere with the upward movement of sap. Tyloses and mycelial fragments jutting into the water column in the vessels would cause a frictional drag and reduce the laminar flow. If, however, vessels are not actively concerned in the ascent of sap, as suggested by Lundegårdh, the presence of tyloses, gums, gels, or gas emboli assume little significance. If the part played by the living cells of the xylem is of considerable importance, their being

affected by the enzymes and/or toxins would seriously interfere with conduction. Moreover, formation of tyloses, gums, or other occluding materials is neither extensive nor universal in occurrence, nor is it specific to the diseased condition. Hence, the importance of these barriers would be either very great or otherwise depending on the exact nature and mechanism by which water is moved upward. The limited significance of the results accruing from fluometric studies brought forward to explain the reduction in water flow in infected stems thus becomes obvious inasmuch as they measure only the turbulent flow of water through the stem under a constant pressure head, which does not even approach the approximate condition in which water flow takes place in living plants. In the present state of knowledge, it is difficult to try to explain precisely the phenomenon of the ascent of sap and on this depends the relative importance of the barriers interfering with the flow of water.

### C. Transpiration—the Dysfunction of Leaf and Stomates

Enhanced transpiration is a feature commonly accompanying pathogenesis. This is primarily due to the removal of the natural protection afforded by the cuticle and by the loss of sensitivity of stomates to respond to changes in leaf water content. Also, an increase in permeability of leaf cells responsible for the availability of water at the evaporating surface tends to increase the rate of loss of water from the leaf tissues. During pathogenesis the increased water loss from the tissues operates as a consequence of one or the other or all of the above mentioned derangements; for instance, *Phaseolus coccineus* inoculated with *Erysiphe polygoni* loses water more rapidly at night and less so during the day than healthy plants, showing that the main factor causing increased water loss is the increased permeability of the host cells. Yarwood (1947) concludes that there is relatively little water loss from the fungus tissue itself or from mechanical openings produced by the pathogen. But in the case of rust infections of *Phaseolus* the rate of transpiration is initially about 70% of the uninoculated during the day but the rate of loss is soon doubled. The nocturnal water loss of rusted turgid bean leaves was greater than that of healthy leaves both before and after the pustules opened. During the day the turgid rusted leaves lost less water than the healthy before the pustules opened, but more afterwards. An analysis of this situation illustrates the behavior of the leaves during disease. Initially, the rate of transpiration of the diseased leaves was lower than that of the checks and this variation was brought about by the differences in their stomatal regulation of transpiration. While the stomata on healthy leaves were wide open, those over rust pustules on the infected leaves were principally closed, thereby causing a lower rate of transpira-



tion; but soon a condition of water deficit developed both in the control and in the rusted leaves as a result of transpiration, and the stomates consequently closed; transpiration decreased and finally became cuticular. The rate of water loss from the infected leaves at this stage was higher than that of control plants, presumably due to the enhanced permeability to water of the injured cuticle over the pustules and to the incomplete closure of the stomates. In general, the rusted leaves continuously lose water more rapidly and dry out sooner without any time for the recovery of internal water deficit. Similar enhanced rates of water loss are known in the case of virus diseases also, e.g., tomato plants infected with tobacco mosaic virus and tomato spotted wilt virus. Changes in the water relations seem to be among the early reactions of the affected cells to virus proteins and the primary effect of the virus appears to be on cuticular transpiration rather than on stomatal behavior (Selman, 1945).

### 1. Leaf Surface Is Reduced

Extensive reduction in leaf area is brought about by various aberrant growth phenomena in leaves due to attack by pathogens. Leaf spot diseases considerably reduce the proportion of healthy leaf cells essential for the development of a suction force adequate to cause a flow of water into the leaves. Other deformities, such as leaf roll, leaf curl, and little leaf, caused by various viruses which induce imbalanced or inhibited growth and expansion of the lamina, also act in the same manner. Defoliation of leaves is a feature accompanying many leaf infections and this seriously interferes with the uptake of water by plants. In addition to reducing the suction force developed by the leaves, such injuries to leaves lead to many far reaching consequences, such as a derangement in the metabolism, starvation due to reduction in the assimilatory tissue, leading to a progressive degeneration and a disturbed auxin balance.

In addition to the external deformities, certain anatomical changes in the affected leaves are brought about as a result of infection in certain cases. Peach leaves infected by *Taphrina deformans* exhibit such a deformity resulting in thickening of leaves. The palisade cells multiply and lose their usual elongated shape and become isodiametric. Structural changes have been noticed in certain virus diseases also, e.g., potato leaf roll, tobacco leaf curl, little leaf of brinjal, etc. (Bawden, 1950). Mesophyll deformation has been observed in cranberries infected by *Exobasidium oxycocci*. In these cases the intercellular spaces are completely obliterated and the leaf becomes stiff. Stiffening is sometimes caused by toxins as in the case of lycopersin action on tomato leaves. The exact manner in which this effect is brought about by toxins is not known.

## 2. Leaf Cells Are Damaged

Damage to leaf cells is caused by both the physical and the chemical action of the parasite.

a. *Necrosis*. Infection takes place either through the stomates or through the cuticle. In the latter case, a rupture is caused either by the pressure of the growing germ tube or by the dissolving action of the enzymes that are secreted by the tip of the advancing germ tube. The growth of the organism inside the leaf tissue may be intercellular or intracellular; in either case, the spread of the organism involves considerable damage to leaf cells. Pectolytic and cellulolytic enzymes are secreted by the spreading mycelium dissolving the cell wall material. The action of these enzymes on the walls of the leaf cells seems to bring about maceration and rotting, culminating in killing of the tissues. In other cases, although such extensive damage to cell walls is not noticed, the protoplasts are killed and their death is accompanied by accumulation of phenolic substances which turn brown on aging and impart that hue to the dead cells. Considerable change in the constitution of the protoplast is brought about by the action of the parasite resulting in various chemical reactions interfering with the utilization of inorganic phosphates. The excretion of phosphorus in the intercellular spaces by cells which normally retain this element is accompanied by an internal secretion of phenolic substances forming coacervates (Humphrey and Dufrénoy, 1944). The coacervate formation affects the metabolism of the cell and the distribution of nucleotides and phosphoproteids with the resultant decompensation of respiration. The latter process may assume various degrees of severity, a mild form permitting the survival of the host cells, while severe damage results in the development of characteristic, hypersensitive, necrotic lesions. In the case of "wildfire" disease of tobacco the affected cells are starved and become devoid of starch and sugars. Oil droplets accumulate and the plastids are disintegrated. Fat soluble yellow carotene pigments, on destruction of chlorophyll, become evident in the yellow halo produced around the spots. The action of the toxin, reported to be functional in this disease, is through competitive inhibition, preventing the utilization of L-methionine (Braun, 1950).

In the case of wilt diseases a different type of injury is discernible. The earliest visual symptom of veinclearing is observed in the case of *Fusarium* wilt of cotton and tomato (Satyanarayana and Kalyanasundaram, 1952; Foster, 1946). In cotton leaves showing veinclearing, histologic examination reveals a disintegration of the plastids in the cells adjoining the veins. It has been suggested that this is caused by the

translocated toxins that permeate the leaf tissues through the veins (Kalyanasundaram, 1954). Similar symptoms have been observed in most virus diseases but the clearing of veins in these instances appears to be due to a suppression of formation of plastids rather than their disintegration (Sheffield, 1938). Long before the appearance of definite vein-clearing symptoms, the veins and veinlets in the leaves exhibit a characteristic fluorescence (Fig. 1) when viewed under ultraviolet light (Subba-Rao, 1954). As stated earlier, the living cells are exposed to the action of fungal  $\beta$ -glucosidases which act on phenolic glycosides inside the cells, liberating conjugated phenols. An injury caused by such liberated phenols results in increased transpiration (Dimond, 1955).

It becomes evident that the changes taking place in the leaves would result not only in increased loss of water due to destruction of forces that retain water against evapo-transpiration, but also in the progressive deterioration of the suction force normally developed in them which, when transmitted to the roots, facilitates absorption.

b. *Permeability Changes.* Depending on the extent of injury to cells, an increase or a decrease in permeability to water occurs. When injury is severe enough to result in death of cells, a marked increase in permeability to both water and solutes occurs. Permeability of protoplasmic membranes is maintained by continued expenditure of energy provided by respiration and any change in respiration affects permeability accordingly. Factors that inhibit growth also inhibit the uptake of water by cells. In the case of plants infected by various parasites, depending on the type of injury each produces, corresponding changes occur in cell permeability to water. An increase in permeability of bean leaves infected by powdery mildew and rust was reported by Yarwood (1947). Increased permeability may be brought about through a direct action on the plasma membrane, resulting in leakage of cell contents. Such a mechanism has been proposed for the action of lycopersamin, one of the wilt toxins produced by *Fusarium lycopersici*. This results in the destruction of osmoregulatory functions of the protoplasts, causing a release of water from the cells. Fusaric acid, another wilt toxin identified in the diseased cotton and tomato plants, increases the permeability of cells to water at low concentrations, although a decrease is observed as the concentration of the toxin increases. Many of the other fungal toxins/antibiotics, e.g., alternaric acid, penicillic acid, patulin, streptomycin, etc, have been shown to impair permeability even at very low concentrations (Gäumann *et al.*, 1952).

The exact manner in which these diverse groups of substances bring about the changes in permeability is not yet known. However, it may be possible to imagine that they act upon one or more of the permeability

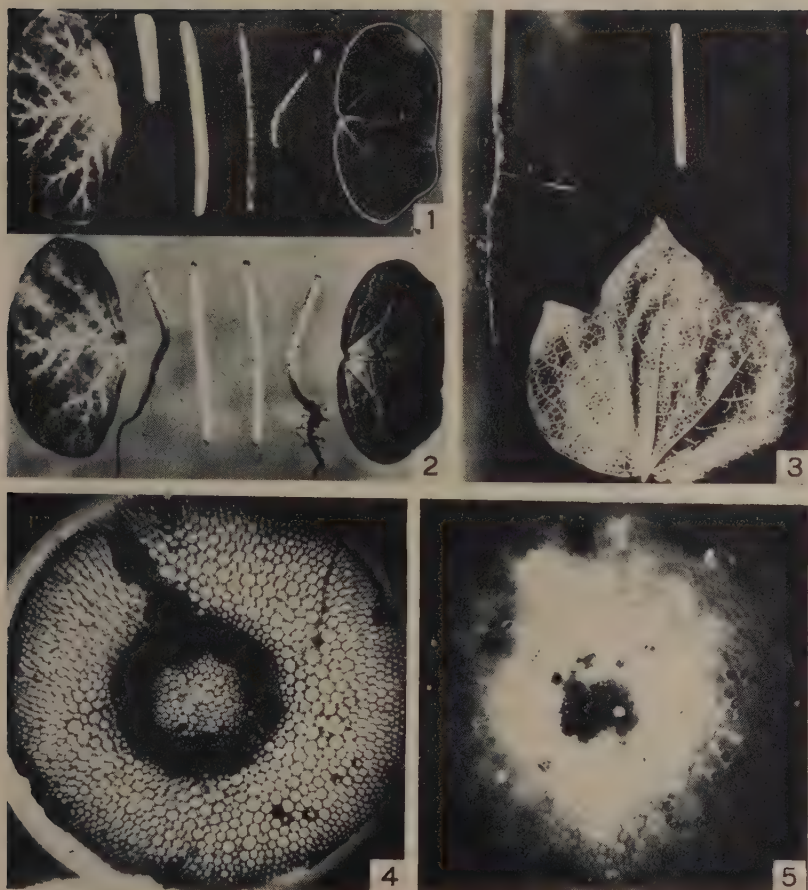


FIG. 1. Symptomatology of cotton wilt: fluorescence phenomenon, (1) Cotyledonary leaf, hypocotyl, and a portion of the stem of cotton seedling infected by *Fusarium vasinfectum* Atk. (left) and the corresponding healthy ones (right) photographed under ultraviolet light. (2) The same specimens as in (1) under incandescent lamp. (3) Ultraviolet light photograph of stem, hypocotyl, and third leaf of an infected cotton plant, the leaves showing distinct vein-clearing symptoms. (4) A transection of the stem of an infected cotton plant showing vascular browning. (5) The same section as in (4) photographed in ultraviolet light. Note the characteristic fluorescence of the vascular region. (From Subba-Rao, N. S. 1954. *J. Indian Botan. Soc.* 33: 443-445. Courtesy of the Editor, *J. Indian Botan. Soc.*)



barriers of a cell, namely, (1) the cell wall, (2) the protoplasmic membrane or plasmalemma, (3) the protoplast itself, and (4) the tonoplast. For instance, cellulolytic and pectolytic enzymes produced locally or transported through the transpiration stream might bring about dissolution of the walls of leaf cells, thereby eliminating or weakening one of the barriers, the effective functioning of which is necessary for maintenance of turgor. The implication suggested here is that when protoplasts are enclosed by rigid walls, their permeability may be decreased or increased, depending on their turgidity (Brouwer, 1954). Polysaccharides and other large molecular carbohydrates, either produced by the parasite or due to breakdown of starch resulting from a condition of water deficit in leaves, may also affect the permeability of leaf cells by blocking the intermicellar capillaries in the cell walls and cause an irreversible impairment to movement of water. The action of substances on the protoplasts causing a change in permeability and water retaining capacity seems to be brought about in many ways, such as an interference with the rates of metabolism that maintain the colloido-chemical properties of the protoplasm, the protoplasmic membranes, synthesis of carrier ions, etc.

c. *Osmotic Changes*. Changes in osmotic pressure result from variations in water content and from variations in solute concentration. The continued loss of water through transpiration, coupled with an inadequate supply of water, produces an increasing water deficit which initiates a chain of reactions, in addition to causing an increase in osmotic pressure. Hydrolytic processes, rather than synthetic processes are stimulated, and this leads to the formation of osmotically active solutes; the accumulation of these in the cells, due to changes in respiratory activity, contributes to an enormous increase in the osmotic pressure of sap in the cells. In some cases, as in the *Fusarium* wilt of cotton, an ionic imbalance in the leaf cells as shown by spectrochemical studies (Sadasivan and Kalyanasundaram, 1956; Sadasivan and Saraswathi-Devi, 1957) and conductivity studies (Gnanam, 1956), has been noticed. The net effect of infection in these cases appears to be an imbalance between monovalent and divalent cations, as indicated by the loss of potassium and accumulation of calcium, manganese, magnesium, etc. (Sadasivan and Kalyanasundaram, 1956; Sadasivan and Saraswathi-Devi, 1957); such an imbalance is known to influence the permeability of cells to water and, consequently, their osmotic pressure (Osterhout, 1916). An ionic imbalance is also known to produce protoplasmic shrinkage, resulting in a water loss (Osterhout, 1956) and this suggests that the toxemic condition produced by many of the wilt toxins probably is preceded by an ionic imbalance, leading to the above changes.

d. *Water Status of Leaf Tissue*. It will be evident from an examination of the sequence of events described above that more water is lost from the leaves than is replenished and the water deficit progressively increases, culminating in the death of cells. A study of the metabolic patterns in the leaves of plants infected with various pathogens reveals the existence of a serious internal water deficit. For instance, an increase in the amount of reducing sugars, nonprotein nitrogen, conductivity of the sap, and respiration has been observed in the *Fusarium* wilt of cotton (Sadasivan, 1957). This reflects a condition of water deficit.

### 3. *Pathological Loss of Water*

The loss of water from diseased plants, which exceeds water intake, is brought about by the disruption of cuticle, dysfunction of stomata, and increased permeability of leaf cells by the action of the pathogen. This excess leads to a progressive decrease in fresh weight, not to mention the other important changes in the vital functions of the plant. Transpiration is essentially a passive process and in the absence of controlling influences, such as those exerted by cuticle, stomata, and the diffusion pressure deficit of leaf cells, would continue according to the evaporating power of the atmosphere. An excess of transpiration over absorption is a phenomenon of daily occurrence in normal healthy plants when conditions favor this process, but soon the controlling effect of the above mentioned factors comes into play and checks its continuation. This does not appear to happen in the diseased plant. However, the loss of turgor and consequent wilting under pathogenesis appear to be brought about, not only by the excess of water loss, but by changes leading to the destruction of osmoregulatory mechanism of the leaf cells and, hence, the rate of transpiration does not seem to indicate all the aspects of pathological wilting. This fact becomes obvious from the experiments where wilting is induced in excised shoots treated with toxin and placed in a saturated atmosphere where the rate of transpiration is negligibly low (Gäumann, 1951). It would thus appear that it is not so much the gain of water by absorption or loss due to transpiration that is of vital importance to plants but the manner in which the plant, at the level of individual cells and tissues, is able to maintain its hydration at the optimum which controls the various metabolic functions and, in turn, is controlled by them.

## V. SYNTHESIS

From a comparative study of various diseases it is clear that a disturbance of the normal water balance occurs at some stage or other, although it is not possible to say whether it is the cause or consequence

of the other phenomena associated with pathogenesis. The general condition of water imbalance in diseased plants is associated with a derangement in its absorption, transport, and/or transpiration and is often accompanied by a disturbance to the various other basic processes, such as carbohydrate and nitrogen metabolism, respiration, and mineral uptake. A study of the metabolic processes that contribute to the maintenance of a normal water balance would, therefore, be necessary in order to understand the principles involved. For instance, the changes in the osmotic pressure of cells and tissues, contributing to the maintenance of a proper gradient facilitating absorption of water and its internal redistribution to various organs to maintain their hydration at an optimum level, depend on the balance between hydrolytic and synthetic processes. These processes, in turn, are influenced in no small measure by the ionic balance in the tissues. It would thus appear that a disturbance in any one of these would initiate a chain of reactions and would lead to an imbalance of various key metabolic functions, and it is very likely that disease arises out of a disturbance to any one of these interrelated processes.

The present state of knowledge, in the absence of a complete picture of the simultaneous changes in all these processes which are linked closely to one another, would at best indicate a correlation rather than a causal relation between the various phenomena accompanying a disease. It is implicit that one should exercise utmost caution in attempting to define the cause of a particular derangement, in the absence of collateral studies of various interrelated processes. It looks as if the constitution of the cell is such that its different reactions would be funneled in a way that would primarily affect its water balance. It is, therefore, reasonable to presume that the primary effect of a disease is on the water status of the cells, which reflects in the various derangements in metabolic functions, leading to a progressive degeneration of vital activities, culminating in death. Thus, it seems to be verily true that "the fire of life burns in water."

#### REFERENCES

- Aberg, B. 1957. Auxin relations in roots. *Ann. Rev. Plant Physiol.* **8**: 153-180.  
Ahrns, W. 1924. Weitere Untersuchungen über die Abhängigkeit des gegenseitigen Mengenverhältnisses der Kohlenhydrate im Laubblatt vom Wassergehalt. *Botan. Arch.* **5**: 234-259.  
Aleksseev, V. A. 1951. Influence of the water regime on the production of auxins and growth of plants. *Doklady Akad. Nauk S.S.S.R.* **81**: 93-96.  
Anderson, D. B., and T. Kerr. 1943. A note on the growth behaviour of cotton bolls. *Plant Physiol.* **18**: 261-269.

- Bawden, F. C. 1950. "Plant Viruses and Virus Diseases," 3rd ed. Chronica Botanica, Waltham, Massachusetts. pp. 335.
- Beckman, C. H., J. E. Kuntz, A. J. Riker, and J. G. Berbee. 1953. Host responses associated with the development of oak wilt. *Phytopathology* **43**: 448-454.
- Benedict, H. M. 1927. Application of Bose's theory of sap rise to ten species of trees. *Am. J. Botany* **14**: 623.
- Bennet-Clark, T. A., A. D. Greenwood, and J. W. Barker. 1936. Water relations and osmotic pressures of plant cells. *New Phytologist* **35**: 277-291.
- Bertelli, J. C. 1948. Histopatología de las lesiones gomosas del Duraznero (*Prunus persica* Sieb. et. Zucc.). *Rev. asoc. ingrs. agron. (Montevideo)* **20**: 9-34.
- Bever, W. M. 1937. Influence of stripe rust on growth, water economy and yield of wheat and barley. *J. Agr. Research* **54**: 375-385.
- Bonner, J., R. S. Bandurski, and A. Millerd. 1953. Linkage of respiration to auxin induced water uptake. *Physiol. Plantarum* **6**: 511-522.
- Bose, J. C. 1923. "The Physiology of the Ascent of Sap." Longmans, Green, New York. pp. 277.
- Braun, A. C. 1950. The mechanism of action of a bacterial toxin on plant cells. *Proc. Natl. Acad. Sci. U. S.* **36**: 423-427.
- Brian, P. W. 1957. Effects of antibiotics on higher plants. *Ann. Rev. Plant Physiol.* **8**: 413-426.
- Brouwer, R. 1954. Water absorption by the roots of *Vicia faba* at various transpiration strengths. III. *Koninkl. Ned. Akad. Wetenschap. Proc.* **C57**: 68-80.
- Chamberlain, D. W., and D. F. McAlister. 1954. Factors affecting the development of brown stem rot of soybean. *Phytopathology* **44**: 4-6.
- Crandall, B. S., G. F. Gravatt, and M. M. Ryan. 1945. Root disease of *Castanea* species and some coniferous and broad leaf nursery stocks, caused by *Phytophthora cinnamomi*. *Phytopathology* **35**: 162-180.
- Davis, D., and A. E. Dimond. 1954. The source and role of phenols in *Fusarium* wilt symptoms. (Abstr.) *Phytopathology* **44**: 485-486.
- Davis, D., P. E. Waggoner, and A. E. Dimond. 1953. Conjugated phenols in the *Fusarium* wilt syndrome. *Nature* **172**: 959-961.
- Dimond, A. E. 1955. Pathogenesis in the wilt diseases. *Ann. Rev. Plant Physiol.* **6**: 329-350.
- Dimond, A. E., G. H. Plumb, E. M. Stoddard, and J. G. Horsfall. 1949. An evaluation of chemotherapy and vector control for combating Dutch elm disease. *Conn. Agr. Expt. Sta. (New Haven) Bull.* **531**.
- Dimond, A. E., and P. E. Waggoner. 1953a. On the nature and role of vivotoxins in plant disease. *Phytopathology* **43**: 229-235.
- Dimond, A. E., and P. E. Waggoner. 1953b. The water economy of *Fusarium* wilted tomato plants. *Phytopathology* **43**: 619-623.
- Dimond, A. E., and P. E. Waggoner. 1953c. The cause of epinastic symptoms in *Fusarium* wilt of tomatoes. *Phytopathology* **43**: 663-669.
- Dixon, H. H. 1914. "Transpiration and the Ascent of Sap in Plants." Macmillan, London. pp. 216.
- Dixon, H. H., and J. Jolly. 1895. On the ascent of sap. *Phil. Trans. Roy. Soc. London* **B186**: 563.
- Farr, C. H. 1924. Cellular interaction between host and parasite. *Phytopathology* **14**: 575-579.
- Foster, R. E. 1946. The first symptom of tomato wilt: clearing of the ultimate veinlets in the leaf. *Phytopathology* **36**: 691-694.



- Gardner, M. W. 1925. Hyperplastic crushing of the tracheal tubes in mosaic tomato stems. *Phytopathology* **15**: 759-761.
- Gaumann, E. 1951. Some problems of pathological wilting in plants. *Advances in Enzymol.* **11**: 401-437.
- Gaumann, E. 1957. Fusaric acid as a wilt toxin. *Phytopathology* **47**: 342-357.
- Gaumann, E., and E. Böhm. 1947a. Über adaptive Enzyme bei parasitischen Pilzen. I. *Helv. Chim. Acta* **30**: 24-38.
- Gaumann, E., and E. Böhm. 1947b. Über adaptive Enzyme bei parasitischen Pilzen. II. *Helv. Chim. Acta* **30**: 1591-1595.
- Gaumann, E., and O. Jürg. 1947. Die physiologischen Grundlagen des parasitogenen Welkens. I. *Ber. schweiz. botan. Ges.* **57**: 3-34.
- Gaumann, E., S. Neeb-Ruch, P. Reusser, and A. Ammann. 1952. Über den Einfluss einiger Welktoxine und Antibiotica auf die osmotischen Eigenschaften pflanzlicher Zellen. *Phytopathol. Z.* **91**: 160-220.
- Gaumann, E., C. Stoll, and H. Kern. 1953. Über Vasinfuscarin, ein drittes Welktoxin des *Fusarium lycopersici* Sacc. *Phytopathol. Z.* **20**: 245-247.
- Gardner, P. 1956. Conductivity studies in cotton plants infected by *Fusarium vasinfectum* Atk. *Proc. Indian Acad. Sci.* **B44**: 125-129.
- Gethyskar, S. S., R. P. Scheffer, J. C. Walker, and M. A. Stahmann. 1953. The role of pectic enzymes in *Fusarium* wilt of tomato. *Phytopathology* **43**: 535-536.
- Gethyskar, S. S., R. P. Scheffer, J. C. Walker, and M. A. Stahmann. 1955. The role of enzymes in the development of wilt of tomato. *Phytopathology* **45**: 381-387.
- Girdler, D. 1944. The mechanism of wilting caused by *Fusarium bulbigenum* var. *lycopersici*. *Phytopathology* **34**: 41-59.
- Greenidge, K. N. H. 1957. Ascent of sap. *Ann. Rev. Plant Physiol.* **8**: 237-256.
- Gregory, F. G., F. L. Middlemore, H. L. Pearce, and H. J. Spencer. 1950. Experimental studies of the factors controlling transpiration. II. The relation between transpiration and leaf water content. *J. Exptl. Botany* **1**: 15-28.
- Griener, B. J. 1941. Studies in the physiology of host-parasite relations. I. The effect of *Bacterium schroeterum* on the water relations of plants. *Proc. Roy. Soc. Victoria [N.S.]* **53**: 268-299.
- Hackett, D. P., and K. V. Thiemann. 1952. The nature of the auxin induced water uptake by potato tissue. *Am. J. Botany* **39**: 553-560.
- Handley, W. R. C. 1939. The effect of prolonged chilling on water movement and radial growth in trees. *Ann. Botany (London)* **3**: 803-813.
- Heath, O. V. S. 1942. Studies in stomatal behaviour. II. The role of starch in the light response of stomata. Part I. Review of literature and experiments on the relation between aperture and starch content in the stomata of *Pelargonium zonale*. *New Phytologist* **43**: 186-211.
- Heldier, R. J. 1956. The loss of substances by cells and tissues (salt glands). In "Encyclopedia of Plant Physiology" W. Ruhland, ed., Vol. 2, Chapter IV. Springer, Berlin. pp. 468-488.
- Hodgson, R., A. J. Riker, and W. H. Peterson. 1947. A wilt inducing polysaccharide from crown gall bacteria. *Phytopathology* **37**: 301-318.
- Hodgson, R., A. J. Riker, and W. H. Peterson. 1949. The toxicity of polysaccharides and other large molecules to tomato cuttings. *Phytopathology* **39**: 47-62.
- Humphrey, H. B., and J. Dufrenoy. 1944. Host-parasite relationship between the oat plant *Avena* spp. and crown rust (*Puccinia coronata*). *Phytopathology* **34**: 21-40.
- Hursh, C. R. 1928. The reactions of plant stems to fungous products. *Phytopathology* **18**: 603-610.

- Husain, A., and A. Kelman. 1957. Presence of pectic and cellulolytic enzymes in tomato plants infected by *Pseudomonas solanacearum*. *Phytopathology* **47**: 111-112.
- Ijlin, W. S. 1957. Drought resistance in plants and physiological processes. *Ann. Rev. Plant Physiol.* **8**: 257-274.
- Jenkins, W. A. 1948. Root-rot disease complexes of tobacco in Virginia. I. Brown root-rot. *Phytopathology* **38**: 528-541.
- Johnston, C. O., and E. C. Miller. 1934. Relation of leaf rust infection to yield, growth and water economy of two varieties of wheat. *J. Agr. Research* **49**: 955-981.
- Kalyanasundaram, R. 1954. Soil conditions and root diseases XIII. Symptomatology of *Fusarium* wilt. *J. Indian Botan. Soc.* **33**: 329-337.
- Kalyanasundaram, R., and C. S. Venkata Ram. 1956. Production and systemic translocation of fusaric acid in *Fusarium* infected cotton plants. *J. Indian Botan. Soc.* **35**: 7-10.
- Kamal, M., and R. K. S. Wood. 1956. Pectic enzymes secreted by *Verticillium dahliae* and their role in the development of wilt disease of cotton. *Ann. Appl. Biol.* **44**: 322-40.
- Kato, J. 1957. *Physiol. Plantarum* (quoted from B. B. Stowe and T. Yamaki. 1957. The history and physiological action of the gibberellins. *Ann. Rev. Plant Physiol.* **8**: 181-216.)
- Kelly, S. M. 1947. The relation between respiration and water uptake in oat coleoptile. *Am. J. Botany* **34**: 521-526.
- Kern, H., and D. Kluepfel. 1956. Die Bildung von Fusarinsäure durch *Fusarium lycopersici* in vivo. *Experientia* **12**: 181-182.
- Kern, H., and B. D. Sanwal. 1954. Untersuchungen über den Stoffwechsel von *Fusarium lycopersici* mit Hilfe von radioaktivem Kohlenstoff. *Phytopathol. Z.* **22**: 449-453.
- Keyworth, W. G. 1953. *Verticillium* wilt of the hop. VI. The relative roles of root and stem in the determination of wilt severity. *Ann. Appl. Biol.* **40**: 344-361.
- Klotz, L. J. 1948. Citrus twig dieback. *Calif. Citrograph* **33**: 381.
- Kramer, P. J. 1933. The intake of water through dead root systems and its relation to the problem of absorption by transpiring plants. *Am. J. Botany* **20**: 481-492.
- Kramer, P. J. 1950. Effects of wilting on the subsequent intake of water by plants. *Am. J. Botany* **37**: 280-284.
- Kramer, P. J. 1951. Causes of injury to plants resulting from flooding of the soil. *Plant Physiol.* **26**: 722-736.
- Kramer, P. J. 1955. Water relations of plant cells and tissues. *Ann. Rev. Plant Physiol.* **6**: 253-272.
- Kramer, P. J. 1956a. Water content and water turnover in plant cells. In "Encyclopedia of Plant Physiology" (W. Ruhland, ed.), Vol. 1, Chapter II. Springer, Berlin. pp. 194-222.
- Kramer, P. J. 1956b. Physical and physiological aspects of water absorption. In "Encyclopedia of Plant Physiology" (W. Ruhland, ed.), Vol. 3, Chapter III. Springer, Berlin. pp. 124-159.
- Kramer, P. J. 1956c. Roots as absorbing organs. In "Encyclopedia of Plant Physiology" (W. Ruhland, ed.), Vol. 3, Chapter III. Springer, Berlin. pp. 188-214.
- Lakshminarayanan, K. 1957. In vivo detection of pectin methyl esterase in *Fusarium* wilt of cotton. *Naturwissenschaften* **44**: 93.
- Lakshminarayanan, K., and D. Subramanian, 1955. Is fusaric acid a vivotoxin? *Nature* **176**: 697.

- Levitt, J. 1951. Frost, drought and heat resistance. *Ann. Rev. Plant Physiol.* **2**: 245-268.
- Levitt, J. 1956. Significance of hydration to the state of protoplasm. In "Encyclopedia of Plant Physiology" (W. Ruhland, ed.), Vol. 3, Chapter VI. Springer, Berlin. pp. 650-651.
- Lineweaver, H., and G. A. Ballou. 1945. The effect of cations on the activity of alfalfa pectinesterase (pectase). *Arch. Biochem.* **6**: 373-387.
- Lineweaver, H., and E. F. Jansen. 1951. Pectic enzymes. *Advances in Enzymol.* **11**: 267-295.
- Linskens, H. F. 1955. Der Einfluss der toxischen Welke auf die Blattausscheidungen der Tomatenpflanze. *Phytopathol. Z.* **23**: 89-106.
- Ludbrook, W. V. 1942. Root amputation experiment with wheat under dry conditions in relation to attack by *Ophiobolus graminis*. *J. Council Sci. Ind. Research* **15**: 121-134.
- Ludwig, R. A. 1952. Studies on the physiology of hadromycotic wilting in tomato plant. *MacDonald Coll. J. Ser. Tech. Bull.* **20**: pp. 39.
- Lundegårdh, H. 1946. Transport of water and salts through plant tissues. *Nature* **157**: 575-576.
- Lundegårdh, H. 1954. The transport of water in wood. *Arkiv Botan.* [2] **2**: 89-119.
- McAlister, D. F., and D. W. Chamberlain. 1951. Water flow through soybean stems infected with brown stem rot. *Plant Disease Reprtr.* **35**: 318-319.
- MacDougal, D. T., J. B. Overton, and G. M. Smith. 1929. The hydrostatic-pneumatic system of certain trees: Movement of liquids and gases. *Carnegie Inst. Wash.* **397**: 99 pp.
- Melhus, I. E., J. H. Muncie, and W. T. Ho. 1924. Measuring water flow interference in certain gall and vascular diseases. *Phytopathology* **14**: 580-584.
- Meyer, B. S. 1956. The hydrodynamic system. In "Encyclopedia of Plant Physiology" (W. Ruhland, ed.), Vol. 3, Chapter VI. Springer, Berlin. pp. 596-614.
- Mostafa, M. A. 1954. Adventitious-root formation by fungal pathogen metabolites as a possible mechanism of disease resistance. *Nature* **174**: 86-87.
- Murphy, H. C. 1935. Effect of crown rust infection on yield and water requirements of oats. *J. Agr. Research* **1**: 387-411.
- Norman, A. G. 1955. The effect of polymyxin on plant roots. *Arch. Biochem. Biophys.* **58**: 461-477.
- Northern, H. T. 1943. Relationship of dissociation of cellular proteins by incipient drought to physiological processes. *Botan. Gaz.* **104**: 480-485.
- Orton, W. A. 1902. *U. S. Dept. Agr. Bur. Plant Industry Bull.* **17**: 9-22.
- Osterhout, W. J. V. 1916. Specific action of barium. *Am. J. Botany* **3**: 481-482.
- Osterhout, W. J. V. 1956. The role of water in protoplasmic permeability and in antagonism. *J. Gen. Physiol.* **39**: 963-976.
- Peirce, G. J. 1934. Observations on sap hydraulics. *Am. J. Botany* **21**: 211-227.
- Peirce, G. J. 1936. Are living cells involved in the ascent of sap? *Am. J. Botany* **23**: 159-162.
- Petrie, A. H. K., and J. G. Wood. 1938. Studies on the nitrogen metabolism of plants. I. Relation between the content of proteins, amino acids and water in the leaves. *Ann. Botany (London)* [N.S.] **2**: 33-60.
- Pierson, C. F., S. S. Gothoskar, J. C. Walker, and M. A. Stahmann. 1955. Histological studies on the role of pectic enzymes in the development of *Fusarium* wilt symptoms in tomato. *Phytopathology* **45**: 524-527.

- Powers, H. R., Jr. 1954. The mechanism of wilting in tobacco plants affected by black shank. *Phytopathology* **44**: 513-521.
- Rabinowitch, E. I. 1945. "Photosynthesis and Related Processes," Vol. I. Interscience, New York. pp. 333-335.
- Richards, L. A., and C. H. Wadleigh. 1952. Soil water and plant growth. In "Soil Physical Conditions and Plant Growth." Academic Press, New York. pp. 73-251.
- Rosene, H. F. 1947. Reversible azide inhibition of oxygen consumption and water transfer in root tissue. *J. Cellular Comp. Physiol.* **30**: 15-30.
- Sadasivan, T. S. 1957. Uptake of ions and metallic chelation in plants. *Proc. Indian Acad. Sci.* **B45**: 1-8.
- Sadasivan, T. S., and R. Kalyanasundaram. 1956. Spectrochemical studies on the uptake of ions by plants. I. The Lundegårdh flame technique in ash analysis of toxin/antibiotic invaded cotton plants. *Proc. Indian Acad. Sci.* **B43**: 271-275.
- Sadasivan, T. S., and L. Saraswathi-Devi. 1957. Vivotoxins and uptake of ions by plants. *Current Sci. (India)* **26**: 74-75.
- Sarmah, K. C. 1956. Tylose formation in tea. *Indian Phytopathol.* **9**: 23-50.
- Satyanarayana, G., and R. Kalyanasundaram. 1952. Soil conditions and root diseases. V. Symptomatology of wilted cotton and red gram. *Proc. Indian Acad. Sci.* **B36**: 54-58.
- Scheffer, R. P., and J. C. Walker. 1953. The physiology of *Fusarium* wilt of tomato. *Phytopathology* **43**: 116-125.
- Scheider, G. W., and N. F. Childers. 1941. Influence of soil moisture on photosynthesis, respiration and on transpiration of apple leaves. *Plant Physiol.* **16**: 565-583.
- Scholander, P. F., W. D. Love, and J. W. Kanwisher. 1955. The rise of sap in tall grape vines. *Plant Physiol.* **30**: 93-104.
- Scholander, P. F., B. Rund, and H. Leivestad. 1957. The rise of sap in a tropical liana. *Plant Physiol.* **32**: 1-6.
- Selman, I. W. 1945. Virus infection and water loss in tomato foliage. *J. Pomol.* **21**: 1-4.
- Sheffield, F. M. L. 1938. Vein-clearing and vein-banding induced by *Hyoscyamus* III disease. *Ann. Appl. Biol.* **25**: 781-789.
- Simmonds, P. M. 1939. Root development in relation to root rots of cereals. *Sci. Agr.* **19**: 475-480.
- Simonis, W. 1952. Untersuchungen zum Dürreeffekt. I. Morphologische Struktur, Wassergehalt, Atmung und Photosynthese feucht und trocken gezogener Pflanzen. *Planta* **40**: 313-332.
- Slatyer, R. O. 1957. The significance of permanent wilting percentage in studies of plant and soil water relations. *Botan. Rev.* **23**: 585-636.
- Sleeth, B. 1933. Relationship of *Fusarium niveum* to the formation of tyloses in melon plants. (Abstr.) *Phytopathology* **23**: 33.
- Smith, F., R. B. Dustman, and C. A. Shull. 1931. Ascent of sap in plants. *Botan. Gaz.* **91**: 395-410.
- Snow, L. M. 1905. The development of root hairs. *Botan. Gaz.* **40**: 12-48.
- Sokoloff, V. P., L. J. Klotz, and F. M. Turrel. 1943. Physiological disturbance in leaves causes mesophyll collapse. *Citrus Leaves* **23**: 8-10.
- Spoehr, H. A. 1919. The carbohydrate economy of cacti. *Carnegie Inst. Wash. Publ.* No. **287**.
- Spoehr, H. A., and H. W. Milner. 1939. Starch dissolution and amylolytic activity of leaves. *Proc. Am. Phil. Soc.* **81**: 31-78.



- Stocker, O. 1948. Beiträge zu einer Theorie der Dürresistenz. *Planta* **35**: 445-465.
- Stocking, C. R. 1956. Hydration and cell physiology. In "Encyclopedia of Plant Physiology" (W. Ruhland, ed.) Vol. 2, Chapter III. Springer, Berlin, pp. 22-37.
- Struckmeyer, B. E., C. H. Beckman, J. E. Kuntz, and A. J. Riker. 1954. Plugging of vessels by tyloses and gums in wilting oaks. *Phytopathology* **44**: 148-153.
- Stubbs, L. L. 1947. A destructive vascular wilt virus disease of broad bean (*Vicia faba* L.) in Victoria. *J. Dept. Agr. Victoria* **45**: 323-332.
- Subba-Rao, N. S. 1954. Fluorescence phenomenon in Fusariase wilt of cotton. *J. Indian Botan. Soc.* **33**: 443-445.
- Subba-Rao, N. S. 1957a. *In vivo* detection of gibberellic acid in 'Foot-rot' infected rice (*Oryza sativa* L.). *Proc. Indian Acad. Sci.* **B45**: 91-94.
- Subba-Rao, N. S. 1957b. Studies in the genus *Fusarium* with special reference to toxicology. Doctoral thesis, Univ. Madras, India.
- Subramanian, D. 1956. Studies on the control of fungal wilts of plants. Doctoral thesis, Univ. Madras, India.
- Tochinai, Y. 1926. Comparative studies on the physiology of *Fusarium lini* and *Colletotrichum lini*. *J. Coll. Agr. Hokkaido Imp. Univ.* **14**: 171-236.
- Van Overbeek, J. 1942. Water uptake by excised root system of tomato due to non-osmotic forces. *Am. J. Botany* **29**: 677-683.
- Waggoner, P. E., and A. E. Dimond. 1954. Reduction in water flow by mycelium in vessels. *Am. J. Botany* **41**: 637-640.
- Waggoner, P. E., and A. E. Dimond. 1955. Production and role of extracellular pectic enzymes of *Fusarium oxysporum* f. *lycopersici*. *Phytopathology* **45**: 79-87.
- Waggoner, P. E., and A. E. Dimond. 1956. Polyphenol oxidases and substrates in potato and tomato stems. *Phytopathology* **46**: 495-497.
- Warne, L. G. G. 1942. The supply of water to transpiring leaves. *Am. J. Botany* **29**: 875-884.
- Wilson, C. C. 1948. Diurnal fluctuations in growth in length of tomato stem. *Plant Physiol.* **23**: 156-157.
- Winstead, N. N., and J. C. Walker. 1954. Production of vascular browning by metabolites from several pathogens. *Phytopathology* **44**: 153-158.
- Yarwood, C. E. 1947. Water loss from fungus cultures. *Am. J. Botany* **34**: 514-520.
- Yu, T. F. 1933. Pathological and physiological effects of *Bacillus tracheiphilus* E.F.Sm. on species of Cucurbitaceae. *Nanking Coll. Agr. Forestry Bull.* [N.S.] **5**: 72.

## Alteration of the Respiratory Pattern in Infected Plants

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### I. INTRODUCTION<sup>2</sup>

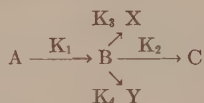
Among the many metabolic changes observed in diseased plant tissue, alteration of respiration is one of the most fascinating subjects. From the biochemical information obtained on this phenomenon, one can gain valuable information about the mechanisms controlling metabolism in plants on one hand, and an elucidation of the underlying principles of the host-pathogen relationship on the other. Respiration occupies a central part of metabolism by providing energy to support cellular processes and is important since the energy from respiratory metabolism is used by the host to carry out its responsive reactions against pathogens. An increase in the respiratory rate is a typical feature of the metabolism of

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<sup>2</sup> The following abbreviations are employed in this chapter: ADP: adenosine diphosphate; ATP: adenosine triphosphate; DNP: 2,4-dinitrophenol; DPN: diphosphopyridine nucleotide; DPN<sup>+</sup>: oxidized diphosphopyridine nucleotide; DPNH: reduced diphosphopyridine nucleotide; H.M.P.: hexose monophosphate; Pi: inorganic phosphate; TCA: tricarboxylic acid; TPN: triphosphopyridine nucleotide; TPN<sup>+</sup>: oxidized triphosphopyridine nucleotide; TPNH: reduced triphosphopyridine nucleotide.

infected plants and has been studied very extensively. Sometimes, when a pathogenic attack on a plant leads to a degenerated condition, the host's rate of cellular catabolism may decline and come to a standstill. Thus, eventually respiration will stop.

In healthy host cells, metabolism progresses in a well-balanced manner. Since all organisms have some flexibility in controlling their metabolism, it can be assumed that they are able to adjust their metabolism to a new circumstance, so that with a slight environmental modification, the over-all reaction will not be altered significantly. However, with a more drastic change of circumstance, such as the presence of a pathogen, the formation of new metabolic pathways may be observed. We can visualize this situation in the sequence below in which A is normally converted through intermediate B to C. If the velocity of reaction  $K_1$  is equal to that of  $K_2$ , then A is connected to C without accumulation of B.



However, if  $K_1$  is proceeding much faster than  $K_2$  or the velocity of  $K_2$  is negligible, then B will accumulate and sometimes new reaction sequences may be induced.  $K_3$  and  $K_4$  indicate those alternative or shunt pathways leading to new abnormal metabolites X and Y. This type of metabolic alteration has been observed in diseased tissues or organs. Many factors affecting this metabolic alteration and varied reactions of host tissue will be surveyed.

In this chapter, four aspects of the alteration of respiration in plant tissues, infected by pathogenic microorganisms, will be discussed. First, we would like to describe the respiratory increase of infected plants. Several biochemical and physiological hypotheses have been put forward to explain it and the information pertaining to this condition in infected plants will be discussed in detail. The Pasteur effect will be discussed in connection with this, since some authors have obtained experimental evidence showing a relationship between the respiratory increase and an inhibition of the Pasteur effect in diseased plant tissue. The possibility that an alternate respiratory enzyme system is activated and participating in the respiratory increase will be considered together with the facts concerning the accumulation of some substances. Second, we shall discuss the H.M.P. pathway. Since this is a recently elucidated pathway for carbohydrate metabolism, few experimental results showing the operation of this pathway in the respiratory pattern of infected plants have been reported. Third, we shall discuss some biochemical phenom-

ena accompanying the respiratory change, such as synthesis of aromatic compounds, metabolism of amino acids, auxin, and lipids, and the physiological function of these phenomena in the host-pathogen relationship. Finally, we shall discuss the relationship between respiratory metabolism of infected host tissue and its defense mechanisms. Unfortunately, this is mostly a speculative field, but it might not be too presumptuous to say that the purpose of the research described in the preceding parts is to elucidate the defense mechanism of the host.

In general, we have tried to point out the important problems for future study rather than describing the scattered reports in this field. Although relatively little work has been done on the physiological and biochemical aspects of plant disease, several articles are helpful in understanding the physiological problems of host-pathogen relationships (Allen, 1953, 1954; Racker, 1954; Walker and Stahmann, 1955; Kern, 1956; Farkas, 1957). The enzymological aspects of plant diseases have been discussed by Farkas and Király (1958); their article contains a considerable number of reports on the oxidative enzymes of infected plants. The alteration of host respiration and its relation to resistance was discussed in a symposium on the "Mechanism of Resistance in Higher Plants," held in Japan in 1956 (Hirai and Suzuki, 1956).

## II. RESPIRATORY INCREASE

### A. Mechanisms Controlling Respiration

Respiratory increase is not a phenomenon uniquely associated with a few plants injured by a specific pathogen, but a kind of general responsive reaction of the plant tissue attacked by pathogenic microorganisms including fungi, bacteria, and viruses. As will be seen in Table I, it has been observed in white potatoes infected by *Penicillium* spp., *Ceratostomella fimbriata*, *Phytophthora infestans*, and in sweet potatoes attacked by *Rhizopus tritici*, *Ceratostomella fimbriata*, or *Helicobasidium mompa*. A similar responsive reaction has also been observed in rice and wheat plants infected by several fungi. It should be emphasized that both obligate and facultative parasites are able to evoke this respiratory increase in host tissue; furthermore, simple chemical treatments or mechanical stimulation is also able to induce an increase in the respiratory rate of plants.

It is necessary to define the term "respiratory increase" which appears frequently in the following discussion. In principle, oxygen uptake per unit weight or volume of infected plant tissue is measured and compared to that of an uninfected plant. In most cases, it is determined for the healthy tissue adjacent to injured tissue; this is then compared to



TABLE I  
EXAMPLES OF THE RESPIRATORY INCREASE IN INFECTED PLANTS

Host	Pathogen	Enzyme or enzyme system studied	Reference
Fungus Pathogens			
Barley leaves	<i>Erysiphe graminis</i>	—	Millerd and Scott (1955, 1956)
Barley leaves	<i>Erysiphe graminis</i>	H.M.P. pathway	Shaw and Samborski (1957)
Cabbage leaves	<i>Botrytis cinerea</i>	Peroxidase	Arzichowskaja (1946)
Cabbage leaves	<i>Botrytis cinerea</i>	Ascorbic acid oxidase Cytochrome oxidase Peroxidase Flavoprotein enzyme	Rubin and Chetverikova (1955)
Cotton stem	<i>Fusarium</i> spp.	Peroxidase Polyphenol oxidase	Stroganov (1947)
Cotton stem	<i>Fusarium vasinfectum</i>	—	Lakshmanan and Venkata Ram (1957)
Potato tuber	<i>Penicillium</i> spp.	Catalase Oxidases	Tombesi (1949)
Potato tuber	<i>Ceratostomella fimbriata</i>	—	Akazawa (1956)
Potato tuber	<i>Phytophthora infestans</i>	Polyphenol oxidase	Rubin and Axenova (1957)
Potato tuber	<i>Phytophthora infestans</i>	Polyphenol oxidase	Tomiya et al. (1957)
Rice plant leaves	<i>Cochliobolus miyabeanus</i>	Polyphenol oxidase	Asada (1957)
Rice plant leaves	<i>Piricularia oryzae</i>	Flavoprotein enzyme	Toyoda and Suzuki (1957)
Safflower hypocotyl	<i>Puccinia carthami</i>	H.M.P. pathway	Daly et al. (1957)
Sweet potato root	<i>Rhizopus tritici</i>	—	Weimer and Harter (1921)
Sweet potato root	<i>Ceratostomella fimbriata</i>	Cytochrome oxidase Peroxidase Polyphenol oxidase	Uritani and Akazawa (1955b)
Sweet potato root	<i>Helicobasidium mompa</i>	Polyphenol oxidase	Suzuki et al. (1957)
Wheat leaves	<i>Erysiphe graminis</i>	—	Allen (1942)
Wheat leaves	<i>Erysiphe graminis</i>	—	Farkas and Király (1955)
Wheat leaves	<i>Puccinia graminis</i>	H.M.P. pathway	Shaw and Samborski (1957)
Wheat leaves	<i>Puccinia graminis</i>	Ascorbic acid oxidase	Király and Farkas (1957)
Bacterial Pathogen			
Tomato hypocotyl	<i>Agrobacterium tumefaciens</i>	Terminal oxidase	Link and Klein (1951)

Virus Pathogens			
Tobacco leaves	Tobacco mosaic virus	—	Owen (1955)
Tobacco leaves	Tobacco mosaic virus	Catalase Peroxidase	Vager (1955)
Tobacco leaves	Tobacco etch virus	—	Owen (1957)
Bean leaves	Tobacco mosaic virus	—	Yamaguchi and Hirai (1956)
<i>Nicotiana glutinosa</i>	Tobacco mosaic virus	—	Yamaguchi (1958)

the respiratory rate for the same tissue of an uninoculated control plant. However, in the case of leaf diseases such as wheat rust, the magnitude of the respiratory rate is often determined by using tissues including both pathogen and infected host tissue, because it is difficult to separate the uninfected from the infected region. The experiments of several workers have shown that the enhancement of the respiratory rate of infected plants can be attributed in part to an independent phenomenon exhibited by host tissue, rather than being due solely to the respiration of the invading microorganism.

In recent years, biochemical investigations of this interesting phenomenon have increasingly received the attention of researchers. Before describing these, it would be valuable to have a general picture from a biochemical point of view of the mechanisms controlling respiration. An excellent review dealing with this subject appeared recently (Laties, 1957). In this article, the author discussed the biochemical principle of the control of rate of respiration, and also tried to interpret several examples of respiratory increase observed in plant tissue by means of a control mechanism. There exist many factors which control the respiratory metabolism of the living cell. They are, for example, concentration of enzymes, substrates, and cofactors, influence of pH, temperature, redox potential, effect of inhibitors; the effect of inhibitors and concentrations of enzymes, substrates, and cofactors will be covered in this section. However, since specific reports are lacking regarding the rest of the topics, they will not be discussed here.

It is believed that the over-all rate of respiration of living organisms is governed by the concentrations of the "pace-makers" in tissue. Recent biochemical studies have shown that these "pace-makers" are the concentrations of ADP and Pi (Laties, 1957; Krebs and Kornberg, 1957). This mechanism will be considered as follows: in the steps of the phosphorylation reaction accompanying the respiratory chain oxidation of DPNH, 3 moles of ATP are generated. Although there is no complete agreement regarding the sites of ATP synthesis from ADP and Pi in the respiratory chain, present knowledge concerning this point will be shown diagrammatically in Fig. 1. Since oxidative and phosphorylative reactions are not



### B. Respiratory Increase of Diseased Plants

The increase in the respiratory rate of higher plant tissues infected with various parasites has been summarized in Table I. Additional data are presented in earlier papers by Sempio (1950) and Allen (1953). Their data are based on measurements of respiration and photosynthesis which were carried out using various infected plants and the generalization was made that the respiratory increase is a characteristic feature of host metabolism. On the contrary, recent work has been much more concerned with the elucidation of the mechanism of the respiratory increase, and is somewhat more biochemical and enzymatic in nature. These newer observations have been selected in Table I.

Research on the respiratory increase of wheat leaves infected with powdery mildew (*Erysiphe graminis*) comprised one of the earliest reports (Allen, 1942). Allen (1953) implied that the respiratory increase of the rusted wheat leaves could be caused by the action of a phytopathogenic toxin which uncouples the oxidative phosphorylation of the host tissue. His excellent article is, so to speak, a milestone in recent biochemical and physiological studies on the respiratory increase in host tissue, and evidently has stimulated many later workers. Some workers have carried out their work to confirm Allen's thought, while others have developed their work starting from his hypothesis. It was then proposed that the active metabolism in the host leading to an increase in the turnover rate of ATP breakdown might also cause the respiratory increase. In his second article, Allen (1954) also extended his idea and suggested that the synthetic processes, accelerated in plants when infected with obligate parasites, may cause the augmented respiration. He did not obtain firm experimental evidence to prove his first hypothesis, but came to this conclusion based on an analysis of Sempio's work (Sempio, 1950). As will be shown, the proposed action of a toxin is like that of DNP which by its uncoupling action will accelerate the breakdown of ATP in host tissue. Consequently, respiratory increase may result. Millerd and Scott (1955, 1956) published two papers concerning the changes in the respiratory rate observed in barley leaves infected with powdery mildew caused by *Erysiphe graminis*. They prepared a crude extract from the infected barley leaves and observed a slight respiratory increase in noninfected leaves when the extract was added. Partially purified extracts also showed a similar effect. Subsequent attempts to obtain evidence of toxin production in rusted wheat and rusted safflower have been unsuccessful (Farkas and Király, 1955; Daly and Sayre, 1957). However, the Hungarian group has just obtained experimental data to support the original hypothesis of a toxin mecha-



nism proposed by Allen. That is, in the leaf tissues of the rust-infected wheat, acid-soluble organic phosphate decreased, concomitant with an increase in Pi (Pozsár and Király, 1958).

One can consider many possibilities for the biochemical action of a phytopathogenic toxin, of which uncoupling would be just one. Let us consider some other aspects of toxin effect in relation to host respiration. The following are examples of toxins or toxin containing extracts which have been isolated from the culture media of pathogens and/or from infected plants. Evaluation of their pathological relation should be made very carefully, as mentioned by several authors, since phytopathogenic toxins isolated from the cultural filtrates of pathogens have not always been found in infected plants (Dimond and Waggoner, 1953; Scheffer and Walker, 1954).

The recent work of Wheeler *et al.* (1958) on the mode of action of a toxin—victorin—produced by *Helminthosporium victoriae* is of extreme interest. These workers examined the effect of victorin on the respiration of tissues of oat varieties. Treatment of root, shoot, or leaf tissues of intact seedlings with victorin resulted in twofold to fivefold increases in the rate of respiration. This response of the plant was detectable after 2 hours and it reached a maximum after 4 to 10 hours. With susceptible oat varieties, victorin caused a respiratory increase 2 to 3 times greater than that caused by DNP. The important fact, however, is that victorin had no effect on the respiration of oat varieties not susceptible to *Helminthosporium victoriae*, whereas DNP affected both types of varieties. Another interesting observation is that homogenates of tissues treated with victorin showed threefold to fourfold increases in the rate of oxidation of ascorbic acid but no corresponding increases in activities of the cytochrome or polyphenol oxidase systems. It appears that the effect of victorin is not due to the uncoupling action but is more complicated.

Tamari and his group studied the rice plant disease caused by *Piricularia oryzae* and succeeded in isolating two toxins from both the culture medium and severely injured plant tissue (Tamari, 1955). One of them,  $\alpha$ -picolinic acid, has a chemical structure similar to that of fusaric acid; the second substance found was named piricularin. Fusaric acid was first isolated from the culture medium of *Gibberella fujikuroi* and identified by Yabuta and his co-workers (1934). Both  $\alpha$ -picolinic acid and fusaric acid inhibit porphyrin-containing enzymes by a chelating action (Tamari and Kaji, 1953). The chemical structure of piricularin has not been conclusively demonstrated, although it has been isolated in a crystalline form. From his observations on the physiological properties of piricularin, Tamari has stated that the mode of action of this toxin is as follows. Polyphenol oxidase which might function as a normal

terminal oxidase in the rice plant is inhibited by piricularin, because it forms a complex with the substrate, chlorogenic acid. Thus, the metabolism of rice plant will consequently be damaged. Relatively high concentrations of piricularin inhibit the respiration of the rice plant ( $1$  to  $4 \times 10^{-4}$  gm. per liter). However, the respiration of plants treated with very dilute solutions ( $1 \times 10^{-6}$  gm. per liter) of this substance shows an increase of 10 to 15%, presumably because of the response of the plant to the toxin, and not because of an uncoupling action of the toxin. As will be discussed in more detail later, the assumption that polyphenol oxidase acts as a terminal oxidase in rice plant should be considered with caution.

Paquin and Waygood (1957) have examined the effect of toxins of *Fusarium oxysporum* f. *lycopersici* on the enzymatic activity of mitochondria in the tomato hypocotyl. At a concentration of  $10^{-2}$  M both lycomarasmin and fusaric acid inhibited the activities of succinic oxidase and cytochrome oxidase, but the inhibition could be completely reversed by the addition of a catalytic amount of cytochrome c. They postulated that the possible effect of lycomarasmin on mitochondria may be in part an alteration of their structural integrity, thus leading to a diffusion of cytochrome c from the particles. They also discussed these observations in connection with the tomato wilt symptoms caused by lycomarasmin. Their opinion is somewhat different from the earlier idea that lycomarasmin destroys the selective permeability of the plasma membrane of the host cell (Gäumann and Jaag, 1947). Fusaric acid has already been shown to have an inhibitory action on the respiration of tomato tissue (Naef-Roth and Reusser, 1954). It appears that frequently a toxin can act as a respiratory inhibitor rather than as an uncoupling agent.

Arzichowskaja (1946) isolated a crude toxin from the cultural medium of *Botrytis cinerea* and showed that the respiration of both infected and healthy cabbage leaves infiltrated with the toxin was augmented. In 1942 it was reported that substances isolated from the culture medium and mycelium of *Gibberella saubinetii* stimulated the respiration of potato tuber (Hellinga, 1942). The author suggested that this substance was likely to be pantothenic acid. Following a similar procedure, Indian workers examined the influence of cultural filtrates of several species of *Fusarium* on the respiration of cotton and observed an increase or decrease of tissue respiration depending on the species of *Fusarium* used (Lakshmanan and Venkata Ram, 1957). Since several compounds, such as organic acids, amino acids, and vitamins, present in the cultural filtrates, have been shown to cause an increase in the respiratory rate of the plants, they assumed that these substances are probably playing a role in stimulating host respiration. In this case, however, the compounds

are very likely accelerating the respiratory rate by providing substrates for respiratory oxidation by the plant.

As a guide for future study on the pathogenicity of microorganisms from the standpoint of respiratory alteration, it is worth while to refer to the elegant work of Pappenheimer (1954) on the diphtheria toxin. He studied the biosynthesis of the toxin in *Corynebacterium diphtheriae* and obtained evidence that it may be a cytochrome component of this bacteria. From his further study on its inhibitory effect on the respiratory system of silkworm, it appears that the pharmacological action of the toxin may be attributable to a blockage of cytochrome synthesis in susceptible animals. According to this mechanism, the toxin would undoubtedly disturb the cell economy of the host either by the total disturbance of the respiratory pattern or by inhibiting oxidative phosphorylation.

It is conceivable that some metabolic substances formed in the infected host tissue may have a biological action more or less similar to that of a toxin produced by pathogens and functioning in the respiratory increase of the host. This possibility has been examined in the case of sweet potato black rot, a disease caused by *Ceratostomella fimbriata*. It has been found that ipomeamarone, a substance produced as an abnormal metabolite of infected host tissue (see Fig. 7), has a stimulating action on the respiration of sweet potato when used in low concentrations (30–50% increase at  $4 \times 10^{-3}$  gm. per liter). Higher concentrations, on the other hand, lower the respiration of sweet potato (50% inhibition at  $4 \times 10^{-2}$  gm. per liter) (Uritani *et al.*, 1954). Since ipomeamarone exerts an uncoupling action on the oxidative phosphorylation of sweet potato mitochondria, the nature of the respiratory increase in sweet potato infected with black rot fungus could be explained by means of an uncoupling action of ipomeamarone. However, analysis for the presence of ipomeamarone has shown that this substance can hardly be detected in the healthy portion of an infected sweet potato, a region where respiratory increase is observed. Therefore, it is difficult to believe that the major portion of the respiratory increase is attributable to the uncoupling action of ipomeamarone. We would rather consider the action of this substance as a side effect in the respiratory increase. The main function of ipomeamarone in the host-pathogen relationship may lie in its fungitoxic nature and necrotic action against the host. The quantity of ipomeamarone accumulating in the infected part of sweet potato is around 10 mg. per gram fresh weight in most cases (Uritani and Akazawa, 1955b). This concentration is sufficient to produce an inhibitory effect on both the host and the pathogen (Table II).

TABLE II  
RESPIRATION AND FORMATION OF POLYPHENOLS AND IPOMEAMARONE  
IN SWEET POTATOES INFECTED WITH *Ceratostomella fimbriata*.<sup>a b</sup>

Variety	Resistance <sup>c</sup>	Respiration <sup>d</sup>		Polyphenols (mg./gm. fresh weight of tissue)	Ipomeamarone (mg./gm. fresh weight of tissue)
		Oxygen uptake ( $\mu$ l./hr.)	% Increase over control		
Norin No. 10	+++	173	70	Not analyzed	Not analyzed
Norin No. 1	++	140	87	4.6	18.9
Chugoku No. 5	++	160	60	3.9	19.8
Nakamurasaki	++	144	55	4.1	14.5
Okinawa No. 100	++	140	118	3.2	9.4
Kanto No. 35	+	134	56	3.6	8.9
Kenroku	+	132	142	4.7	9.4
Suigen	—	123	45	4.3	9.8
Norin No. 5	—	88	67	4.4	5.6

<sup>a</sup> After Uritani and Akazawa (1955b). Reproduced from Kagaku.

<sup>b</sup> Sweet potatoes were sliced (1–2 cm. thick), and some of the slices were inoculated with a spore suspension of *Ceratostomella fimbriata*: the rest served as a control sample. They were subjected to further analysis.

<sup>c</sup> The number of plus signs represents the degree of resistance of sweet potato against the pathogen in a decreasing order. The minus sign represents a susceptible variety.

<sup>d</sup> After 48 hrs. inoculation of the fungus at 25°C., 20 slices, 0.5 mm. thick and 7 mm. in diameter each, were prepared from the healthy tissue adjacent to the infected region, and respiratory oxidation was measured manometrically at 30°C. Sound tissue was obtained from the control sample and its respiratory activity was compared with that of the infected one.

Furthermore, there are two other possibilities bearing on the uncoupling type respiration in infected plants. Since activity of oxidative phosphorylation is closely associated with the biochemical structure of mitochondria, and since in some pathological circumstances plant mitochondria might be destroyed, respiratory oxidation would not be coupled to phosphorylation. A second possibility is the operation of an alternative respiratory pathway other than the TCA cycle-cytochrome system which does not link to the phosphorylation reaction. Experimental findings concerning the activation of this system are discussed in the following section; however, its relation to oxidative phosphorylation has not been studied thoroughly.

Another explanation for the mechanism of respiratory increase may be found in the active metabolism accelerating the ATP-utilizing reactions of host tissue, as suggested by several authors (Allen, 1954; Akazawa and Uritani, 1955; Tomiyama and Takase, 1956; Suzuki *et al.*, 1957). Many anabolic processes requiring energy undoubtedly accelerate



ATP breakdown and consequently increase the respiratory rate. In support of the above mechanism, many characteristic features indicating activation of the metabolism of the host have been reported such as (1) accumulation, mobilization, and synthesis of phosphorus and carbon compounds, (2) growth of the host tissue, (3) synthesis of proteins including activation of enzymes and enzyme systems, and (4) increase in protoplasmic work, such as protoplasmic streaming (cyclosis). The over-all picture of these events is diagrammatically shown in Fig. 2.

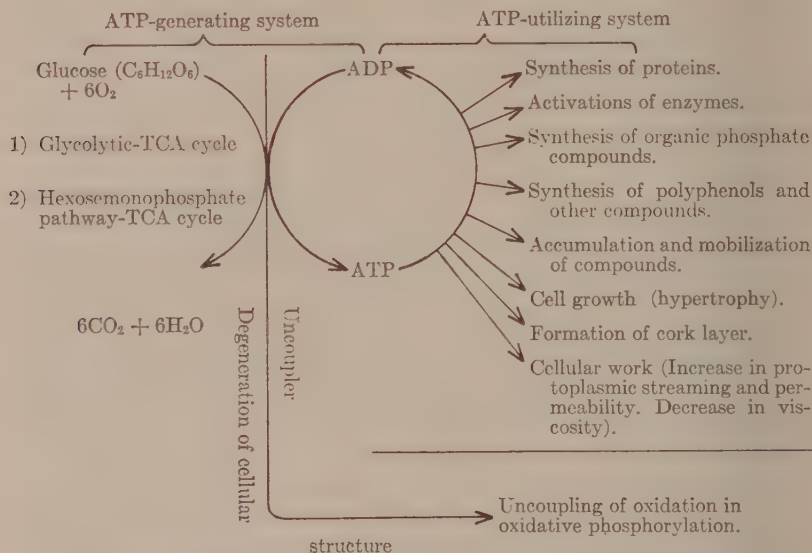


FIG. 2. Relationship between respiratory increase and ATP-utilizing systems in infected plants.

The first case has probably been studied most extensively and many articles on this subject using radioisotopic techniques have been published. Several workers have observed the accumulation of  $P^{32}$  in the wheat leaves infected with rust (Gottlieb and Garner, 1946; Yarwood and Jacobson, 1955). Yarwood and Jacobson (1950) have also reported selective absorption of  $S^{35}$  by the infected leaves. Recently, this problem has been investigated by Canadian workers on a large scale (Shaw *et al.*, 1954; Shaw and Samborski, 1956). They have examined the assimilation of  $C^{14}O_2$ , the uptake and the distribution of  $P^{32}$  and other radioactive organic substances—including carbohydrates, organic acids, and amino acids around the spots on the cereal leaves infected with rust

and mildew. The accumulation of glucose-1-C<sup>14</sup> occurs in the host tissue at the site of the fungus colonies and parallels the increase of respiration. Interestingly, the accumulation was not observed in the tissue of the host killed by the pathogens. This accumulation was also inhibited by anaerobiosis and by some phosphorylative and respiratory inhibitors like DNP, azide, or H<sub>2</sub>S. The authors came to the conclusion that the accumulation and mobilization of carbohydrates and organic and amino acids under pathological conditions are active processes closely connected with the respiration of the host. Net synthesis of organic phosphorus compounds has been observed in sweet potato infected with either *Ceratostomella fimbriata* or *Helicobasidium mompa* (Akazawa and Uritani, 1955; Suzuki *et al.*, 1957).

The work of Daly and Sayre (1957) is of interest for the discussion of case number 2. They have studied the relationship between cellular growth of the host and the increase in the respiratory rate of safflower infected with *Puccinia carthami*. During the vegetative development of the pathogen, the diseased hypocotyl elongated approximately twice as rapidly as did the healthy hypocotyl and showed a greater absolute length and weight with a concomitant doubling in the host respiration. They concluded that the abnormal growth of the rusted host demands energy, thus accelerating ATP breakdown and eventually enhancing the respiratory rate of the host. This assumption eliminates the need for an uncoupling mechanism by a toxin. Accordingly, they failed to get evidence of toxin production. These authors emphasized the importance of study on the hormonal control of both the growth and the respiratory metabolism of the host. A recent study on the physiology of host-pathogen relationships using rusted wheat deserves detailed description (Samborski and Shaw, 1956). A few of their experimental results are shown in Figs. 3 and 4. In the case of susceptible species (Little Club), but not of resistant species (Khapli), of wheat infected with rust, hypertrophy was observed with a respiratory increase indicating the unbalanced elongation of the host cells under pathogenic infection. Perhaps we can invoke a mechanism similar to that of Daly and Sayre for a possible explanation of the respiratory increase in rusted wheat. Localized hypertrophy is frequently observed in hosts infected by obligate parasites (Yarwood and Cohen, 1951).

There are several reports on protein synthesis and on the activation of enzyme systems in infected plants and we would like to discuss here the former subject in relation to case number 3. In sweet potato infected with *Ceratostomella fimbriata*, Akazawa and Uritani (1955) have observed the apparent synthesis of proteinous nitrogen with a concomitant increase in respiration. Tomiyama and his associates (1955, 1956) have

also demonstrated the fact that in potato tissue infected by *Phytophthora infestans*, a notable increase in water-soluble protein occurs. A similar result has been reported in sweet potato infected with *Helicobasidium mompa* (Suzuki *et al.*, 1957). Activation of enzymes and/or enzyme systems to be discussed later also demands energy, consequently, respiratory rate may increase.

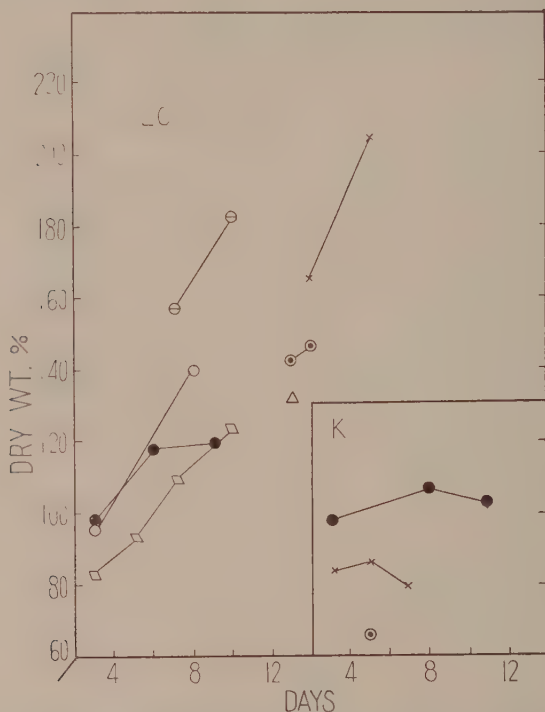


FIG. 3. The change in dry weight after rust infection of the first leaves of Little Club (LC) and Khapli (K) wheat species. Ordinate: dry weight of 60 leaf-discs (diameter 2.8 mm.) as a percentage of the weight of discs cut from uninfecting leaves. Abscissa: days after inoculations. Each symbol represents a different experiment. (After Samborski and Shaw (1956), Reproduced from Canadian Journal of Botany).

Recent biochemical knowledge regarding the climacteric respiratory increase of fruits is pertinent to this problem. It has been generally observed that the respiratory rate of fruit tissue gradually increases in its ripening process and reaches its maximum value just before complete ripening. Analysis of nitrogenous compounds in parallel with determina-

tions of the respiratory rate have been carried out during apple ripening (Robertson and Pearson, 1954; Hulme, 1955). Both groups have demonstrated the pronounced increase of protein content in fruit at the onset of the respiratory climacteric. Robertson's group has also noticed a slight response of tissue respiration to DNP at the climacteric stage. Their conclusion is that the climacteric respiratory increase is caused by

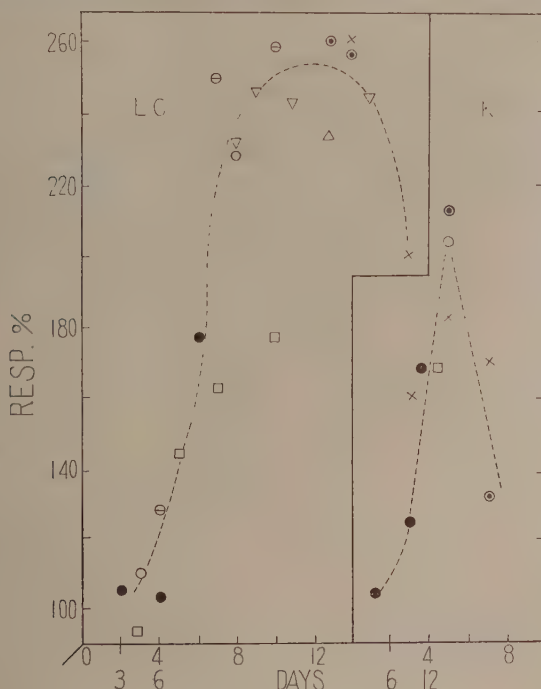


FIG. 4. The change in respiration after rust infection of the first leaves of Little Club (LC) and Khapli (K) wheat species. Ordinate: oxygen consumption per 10 mg. dry weight per hour as a percentage of the value for uninfected leaves. Abscissa: days after inoculation. Symbols: as for Fig. 3. (After Samborski and Shaw (1956). Reproduced from Canadian Journal of Botany).

a net synthesis of protein requiring energy and that this accelerates the turnover rate of ATP breakdown. This hypothesis is in striking contrast to that of an uncoupling one for the climacteric rise of avocado proposed by Millerd *et al.* (1955).

As to case number 4, all protoplasmic work demands energy. Increases in protoplasmic streaming and permeability and viscosity decreases which have been observed in infected plants may play a part in



the respiratory increase (Thatcher, 1942, 1943; Allen, 1953; Tomiyama, 1954, 1955a; Greenham and Müller, 1956). Tomiyama observed (by means of a microscopic technique) an increase in the protoplasmic streaming of the midrib cells of potato when the plant is infected by *Phytophthora infestans*.

To correlate the above mentioned metabolic events with the present hypothesis that respiratory increase is caused by a higher turnover rate of reactions utilizing ATP, we have to ascertain the concentrations of ADP and Pi and/or determine the ATP:ADP ratio in infected plant tissue as well as clarify the augmented activity of the ATP-utilizing systems of the host. Since ATP and ADP are probably existing in steady state concentrations, the ratio ATP:ADP will be altered in response to an environmental change. It will be necessary to determine this ratio cautiously and the following two technical points should be kept in mind: (1) the analytical methods for determining absolute amounts of ATP and ADP are not very sensitive, and (2) there has not been any appropriate procedure for stopping instantaneously the metabolism of plant tissue. In order to substantiate the enhanced activity of ATP-utilizing systems, the following projects would be of great value: (1) elucidation of the intermediary metabolism of phosphorus using  $P^{32}$ , (2) clarification of the nature of synthesized protein, and (3) demonstration of the biochemical nature of cellular work. The experiments conducted by Loughman and Martin (1957) and Loughman and Russel (1957) are germane for the discussion of (1). The incorporation of  $P^{32}$  into various organic phosphorus compounds in intact barley roots was investigated by using paper chromatography and a sensitive recording counter. After short periods of phosphate absorption, an intensive uptake of  $P^{32}$  into the acid soluble phosphorus fraction which contains nucleotides was observed. This process was inhibited by DNP. There were differences in the distribution of phosphorus into the various fractions depending on the concentration of  $P^{32}$  supplied, but eventually they showed the status of phosphate metabolism of the plant tissue in the steady state condition. Application of this procedure would be of great help in elucidating the phosphate metabolism of infected plant tissue in conjunction with the respiratory increase.

Demonstrating an effect on respiration due to DNP is very frequently used to show that the phosphorylative reaction is the limiting "pace-maker" in tissue respiration (Beevers, 1953). Exogenous addition of DNP to tissue will cause an extra output of respiratory oxygen, depending on the velocity of ATP-utilizing reactions in the cells being studied. Akazawa and Uritani (1955) have surveyed the DNP effect on the respiration of the sweet potato infected with *Ceratostomella fimbriata* and compared

the magnitude of respiratory increase with that of uninfected plants. Two to four days after inoculation, when the respiratory increase showed a maximum value, the percentage increase of respiration due to DNP addition was considerably smaller than in the healthy control tissue. At a later stage, when the respiratory increase of the infected tissue declined, the DNP effect was almost the same as that in control tissue. Other workers using wheat seedlings infected with rust have observed a decreased respiratory rate in the DNP treated tissues in comparison with a positive DNP response in the healthy tissue (Király and Farkas, 1956). In the case of the rusted wheat studied by Shaw and Samborski (1957), a decreased percentage response to DNP was found in the infected tissue, although the actual response was the same with both infected and uninfected tissues. However, it should be emphasized that a weak response of host respiration to added DNP per se does not distinguish between the two alternative mechanisms of a rapid breakdown of ATP in cells either by (a) an uncoupling phenomenon or by (b) active metabolism which we are discussing now.

### *C. The Inhibition of the Pasteur Effect*

In the physiological study on the rusted wheat leaves, Sempio (1950) noticed the Pasteur effect abolishment which concomitantly occurred with the respiratory increase. As described before, Allen (1953) analyzed Sempio's data and proposed a mechanism for the respiratory increase by means of a Pasteur effect inhibition.

Generally, the Pasteur effect is defined as the inhibition of fermentation in living organisms under aerobic conditions. It seems reasonable to extend the definition of this phenomenon to include reduction of carbohydrate breakdown under aerobic conditions with slight formation of a fermentative product like ethanol or lactic acid. Therefore, the abolition of the Pasteur effect will be considered to be a release of the suppression of aerobic carbohydrate breakdown as compared to the anaerobic one. Many hypotheses have been proposed to interpret the Pasteur effect (Dickens, 1951). According to Johnson (1941) and Lynen and Königsberger (1951), the Pasteur effect would be interpreted as follows: due to the effective operation of oxidative phosphorylation more ATP is made available via the aerobic breakdown of carbohydrate than is formed under anaerobic conditions. This results in a decrease in the concentrations of ADP and Pi which influences the activity of the glyceraldehyde dehydrogenase system requiring ADP, Pi, and DPN. This enzyme is believed to be one "pace-maker" of glycolysis and a decrease in the activity of this enzyme system will subsequently decrease the rate of operation of the glycolytic pathway. The finding that uncoupling agents

such as DNP and *p*-nitrophenol inhibit the Pasteur effect supports the hypothesized mechanism of Johnson and Lynen. Australian workers obtained favorable data that the Pasteur effect in plant tissue can be regulated by the concentrations of the phosphate acceptor system (Rowan *et al.*, 1956). Provided this mechanism operates *in vivo* unequivocally, the Pasteur effect will be inhibited by any metabolic event which will increase the concentrations of ADP and Pi, either by the uncoupling or by the increased rate of cellular reactions requiring ATP. This is, therefore, very closely connected with the mechanism of increase in the respiratory rate.

Allen's idea is that some toxins secreted by the pathogens might cause the abolishment of the Pasteur effect by their uncoupling action, subsequently increasing the respiratory rate. Farkas and Király (1955) have proposed that the inhibition of the Pasteur effect is probably related to the respiratory increase in wheat leaves infected with rust and mildew. In this case, they assumed that it might be inhibited by the uncoupling mechanism, but were unsuccessful in attempting to isolate a phytopathogenic toxin. Similarly, Japanese workers have shown that the abolishment of the Pasteur effect in sweet potato (especially in the case of a susceptible variety) infected with *Helicobasidium mompa* is associated with a respiratory increase (Suzuki *et al.*, 1957). American workers have obtained similar experimental evidence for an inhibition of the Pasteur effect in rust infection of safflower hypocotyl (Daly and Sayre, 1957; Daly *et al.*, 1957). The Canadian group has also noticed a marked reduction or perhaps absence of the Pasteur effect concomitant with the stimulated respiratory rate of rusted wheat (Shaw and Samborski, 1957).

In the field of plant physiology very often a ratio of anaerobic to aerobic carbon dioxide output

$$Q_{\text{C}\text{O}_2}^{\text{N}_2} : Q_{\text{C}\text{O}_2}^{\text{O}_2}$$

larger than 0.33 is considered to indicate the existence of the Pasteur effect in the organ or tissue examined (Turner, 1951; James, 1953a). In both American and Canadian reports, the ratios of the infected rusted plant tissues fell after inoculation to below 0.3 at the final stage of the infection as compared to those for uninfected plants which do not decrease markedly from a value of 0.5 to 0.6. There was no increased anaerobic carbon dioxide formation accompanying the pathogenically increased aerobic carbon dioxide formation. From these observations, they assumed the predominant participation of the H.M.P. pathway in the respiratory pattern of infected plants and obtained supporting experimental evidences as described in the next section. The predominant participation

of this pathway in the infected plant tissue would reduce the numerical figure of

$$Q_{\text{CO}_2}^{\text{N}_2} : Q_{\text{CO}_2}^{\text{O}_2}$$

and may explain the abolition of the Pasteur effect.

It has been shown recently that the Pasteur effect occurs when mitochondrial preparations exist together with a glycolytic system (Aisenberg and Potter, 1957; Aisenberg *et al.*, 1957). The data of these workers suggest that suppression of the carbohydrate breakdown might not be regulated by the concentrations of ADP and Pi, but by some unstable system existing in a steady state form in the oxidized mitochondria. This system may be inhibiting hexokinase or phosphofructokinase. Providing this mechanism operates *in vivo*, carbohydrate breakdown could be regulated by "oxidized mitochondria" rather than by the concentration of ADP in living organisms. This hypothesis is reminiscent of the theory of Engelhardt and Sakov (1943) on the mechanism of the Pasteur effect, which was proposed 15 years ago. According to these workers, phosphofructokinase is very sensitive to oxidizing agents generated in respiratory oxidation. It would be interesting to investigate in diseased plants the mechanism of abolition of the Pasteur effect in terms of these theories.

None of these hypotheses can thoroughly explain abolition of the Pasteur effect in diseased plants. In addition to the determination of the

$$Q_{\text{CO}_2}^{\text{N}_2} : Q_{\text{CO}_2}^{\text{O}_2}$$

ratio, more detailed analytical data are necessary before the phytopathological role of the Pasteur effect can be fully understood, e.g., the consumption of the carbon source and formation of fermentation products.

#### D. Activation of Enzyme Systems

Probably, the concentration of enzymes and their substrates is affecting the over-all rate of respiration. Many experiments have been published dealing with the activation or alteration of respiratory enzymes with particular reference to terminal oxidases in infected plants. Frequently, some specific substances such as polyphenols or ascorbic acid have also been found to accumulate in infected host plants. From these observations, we are inclined to assume that these anomalous metabolic events may somehow be concerned with the mechanism of respiratory increase. First we would like to discuss some general aspects of terminal oxidases in plants.

What constitutes the normal terminal respiratory oxidase in higher plants is still debated. It is generally believed, however, that the terminal



electron transport system of living organisms, including plants, is mediated by the cytochrome system. Polyphenol oxidase, ascorbic acid oxidase, and some other soluble oxidases are said to function in the respiratory pattern of certain plant organs at a specific stage of their development. Mostly, such claims rest on (1) experimental results using specific inhibitors of respiratory oxidation on homogenates or tissues, or (2) failure to demonstrate cytochrome oxidase activity in the plant systems examined. Since the evaluation of these techniques has been discussed by several authors (James, 1953a, b; Hill and Hartree, 1953; Bonner, 1957), a detailed description is omitted and basic points will be emphasized. At the present time there are two appropriate methods available for distinguishing between cytochrome oxidase and the copper enzymes (e.g., ascorbic acid oxidase and polyphenol oxidase); either by testing the photoreversibility of carbon monoxide inhibition of each enzyme, or by examining the potentiality of oxidative activity under various tensions of oxygen. In the first method, carbon monoxide inhibition of cytochrome oxidase is completely reversed by light whereas with copper enzymes it is not. (It has been occasionally reported that carbon monoxide does not inhibit ascorbic acid oxidase.) In this case, transparency of tissue to light should be carefully checked. As to the second method, the affinity of cytochrome oxidase for oxygen is very high—that is, it functions normally even at low oxygen tension. On the other hand, copper enzymes have a low affinity for oxygen. Here the factor of the rate of oxygen diffusion should be kept in mind, and low activity of tissue respiration under low oxygen tension per se does not necessarily indicate the functioning of a copper enzyme. By using the above techniques, Thimann's group has clearly shown the sole function of cytochrome oxidase in the respiration of the pea internode (Eichenberger and Thimann, 1957), although ascorbic acid oxidase activity is very high in this tissue. This result is in striking contrast to the developmental study of barley roots, in which ascorbic acid oxidase has been reported to progressively replace cytochrome oxidase during the first 7 days of root development (James and Boutler, 1955).

There have been many reports concerned with the activation of copper-containing enzymes in infected plant tissues (polyphenol oxidase and ascorbic acid oxidase), but unfortunately, none of them has been performed by carefully considering the above problems. A few examples cited hereafter will be of some value for further discussion. Király and Farkas (1957) have examined the respiratory alteration of the wheat seedlings infected with stem rust, and from experimental results using specific substrates and inhibitors have come to the conclusion that the respiratory pattern of the plants shifts drastically to an ascorbic acid

oxidase system shortly after the infection. Although this is an interesting observation, more detailed information will be necessary before a final conclusion can be reached. Uritani and Iechika (1953) have shown the accumulation of large amounts of ascorbic acid in sweet potato tissue infected by *Ceratostomella fimbriata*. Beevers (1954) has observed the oxidation of DPNH via an ascorbic acid oxidase system in cucumber; a survey of this system would be interesting in connection with the above findings.

In the case of sweet potato infected with the black rot fungus, activation of cytochrome oxidase has been confirmed in cell-free systems (Uritani and Akazawa, 1953). However, from the experimental results of carbon monoxide inhibition and its photoreversibility in the respiration of the infected sweet potato, a part of the terminal oxidase appears likely to be mediated by a noncytochrome system such as polyphenol oxidase (Uritani *et al.*, 1955). Since there is a possibility that the infected sweet potato slices are not transparent enough to light, this is not a conclusive result. Polyphenol oxidase is a widely occurring oxidase in the plant kingdom, and its activity is particularly augmented under pathological conditions. Rubin's group in Russia put forward the idea of an alteration and activation of specific respiratory enzymes from their extensive work on the physiology of host-pathogen relationship. One example is that potatoes infected with *Phytophthora infestans* show a considerable increase in the activity of polyphenol oxidase and the amount of polyphenols (Rubin *et al.*, 1947; Rubin and Axenova, 1957). They feel that this oxidase plays a significant role in the respiratory pattern of the diseased plant and in the immunity of the plant (Rubin *et al.*, 1955). Another example is the study of cabbage infected by *Botrytis cinerea*, in which the enhancement of peroxidase activity was observed in striking contrast to the complete impairment of other respiratory systems such as cytochrome oxidase and ascorbic acid oxidase (Rubin and Chetverikova, 1955). An interesting fact is that a similar metabolic disturbance can be induced by the *Botrytis* toxin obtained from culture filtrates. From these observations, it was proposed that either *Botrytis* infection of cabbage or the toxin per se might switch the respiratory pattern of the host to a flavoprotein system, which will produce hydrogen peroxide by the oxidation of the substrate. The hydrogen peroxide produced by the operation of the new pathway is presumably broken down and removed by means of peroxidase. Both the role of the flavoprotein enzyme and the function of peroxidase in the terminal respiratory pattern of cabbage are totally unknown at the present time. Rubin himself does not as yet have any experimental evidence that a flavoprotein enzyme is actually activated and is functioning in the *Botrytis*-infected cabbage.

This attractive hypothesis must, therefore, be proven by future study. Activation of a flavoprotein enzyme in rice plants (in the case of a susceptible variety) infected by *Piricularia oryzae* has been reported by Toyoda and Suzuki (1957).

In conclusion, the authors' opinion is that there have not been any thorough supporting data for the participation of a noncytochrome system (polyphenol oxidase, ascorbic acid oxidase, peroxidase, or flavoprotein) in the pathogenically induced respiratory increase of plant tissue. It is highly desirable to get more data on this problem. At the same time, it is important to clarify the activation of enzymes or enzyme systems other than terminal oxidases, for example, those of the TCA cycle, and the glycolytic and the H.M.P. pathways.

### III. THE HEXOSE MONOPHOSPHATE PATHWAY

During the course of study on respiratory increase, experimental evidence accumulated which pointed to the operation of a new pathway, the H.M.P. pathway, in the respiratory pattern of infected plants. For instance, the following characteristic features were noticed in the respiration and carbohydrate metabolism of infected plant tissue: (1) insensitivity of tissue respiration to malonate, a competitive inhibitor of succinic dehydrogenase, thus ruling out the stimulation of operation of the TCA cycle (Farkas and Király, 1955; Heitefuss, 1957); (2) a relatively weak response of increased respiration to NaF, the magnitude of NaF sensitive respiration being the same between infected and uninfected plant tissues, which may indicate the participation of a nonglycolytic pathway in the augmented respiration (Daly *et al.*, 1957); and (3) a remarkably low value for the ratio in infected tissue,

$$Q_{\text{CO}_2}^{\text{N}_2} : Q_{\text{CO}_2}^{\text{O}_2}$$

indicating the apparent absence of a Pasteur effect (Shaw and Samborski, 1957; Daly and Sayre, 1957). However, the magnitude of anaerobic CO<sub>2</sub> evolution remains approximately the same between infected and uninfected tissues as has already been discussed in C of the previous section.

Both American and Canadian workers succeeded independently in obtaining evidence for the operation of the H.M.P. pathway in rusted safflower and rusted wheat or mildewed barley (Daly *et al.*, 1957; Shaw and Samborski, 1957). Noticeable evolution of C<sup>14</sup>O<sub>2</sub> from glucose-1-C<sup>14</sup> by rusted wheat leaves had been already demonstrated by the latter group (Shaw and Samborski, 1956). Measurement of the so-called C-6:C-1 ratio by both groups showed the predominant participation of the H.M.P. pathway in the infected plant tissues. Here it would be help-

ful to describe the general sequence of the H.M.P. pathway which is diagrammatically shown in Fig. 5 together with the glycolytic pathway (Krebs and Kornberg, 1957).

In the first step of the H.M.P. pathway, glucose-6-phosphate is dehydrogenated in the presence of TPN by Zwischenferment or glucose-6-phosphate dehydrogenase, and 6-phosphoglucono- $\delta$ -lactone is formed. In the following step, 6-phosphogluconate, which is formed from the

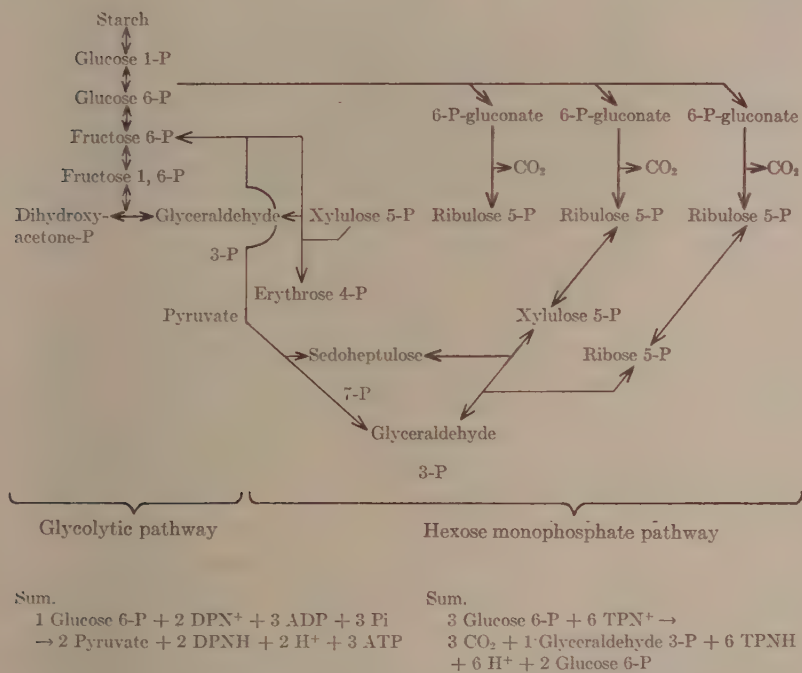


FIG. 5. The glycolytic and hexose-monophosphate pathways.

phosphoglucono-lactone by the action of lactonase, is decarboxylated oxidatively also in the presence of TPN to give a pentose phosphate. Eventually this pathway ends up by either forming phosphoglyceraldehyde, which is metabolized via the glycolytic pathway, or by condensing two moles of triose phosphate to regenerate a hexose phosphate which can re-enter the H.M.P. pathway. As would be expected, if glucose is catabolized via the glycolytic pathway, CO<sub>2</sub> initially comes off equally from position C-3 and C-4 of the glucose molecule, and lastly from C-1 and C-6 again with equal activity. On the contrary, if glucose is oxidized



via the H.M.P. pathway, the C-1 position will contribute most to the evolved  $\text{CO}_2$  and C-6 the weakest during short term experiments. Therefore, if we feed the same amount of glucose-6- $\text{C}^{14}$  and glucose-1- $\text{C}^{14}$  to a tissue and determine the ratio of evolved radioactive  $\text{CO}_2$  in each case (C-6:C-1), it will show the magnitude of relative participation of the glycolytic and the H.M.P. pathways. This procedure was originally introduced by Bloom and Stetten (1955) and later widely applied in investigating the carbohydrate catabolism and the respiratory pattern of various organisms (Beevers and Gibbs, 1954a, b; Axerlod and Beevers, 1956). The C-6:C-1 ratio equaling 1.0 indicates the sole participation of the glycolytic pathway in tissue and a ratio smaller than 1.0 may indicate the operation of the H.M.P. pathway. Numerically, the percentage participation of the H.M.P. pathway or the glycolytic pathway can be calculated by various ways. Shaw and Samborski (1957) calculated the fraction of glucose catabolism via the glycolytic pathway according to the following equation (A), based on the fact that C-1 and C-6 carbon atoms come off last when glucose is oxidized

$$A = \frac{\text{c.p.m. from UL} - (\text{c.p.m. from C-1} - \text{c.p.m. from C-6})}{\text{c.p.m. from UL}}$$

(c.p.m. from UL, C-1 and C-6 denote the radiochemical yield of  $\text{C}^{14}\text{O}_2$  from the fed substrates of uniformly labeled glucose, glucose-1- $\text{C}^{14}$ , and glucose-6- $\text{C}^{14}$  respectively) by the glycolytic pathway. Korkes (1956) estimated the percentage participation of the H.M.P. pathway and the glycolytic pathway, respectively, according to the following equations (S and G), G being between C-6:C-1 and A.

$$S = \frac{1 - R}{1 + 5R}, \quad G = \frac{6R}{1 + 5R}$$

(S = fraction of  $\text{CO}_2$  via the H.M.P. pathway. G = fraction of  $\text{CO}_2$  via the glycolytic pathway. R = C-6:C-1.)

The data of Daly and his associates show that the C-6:C-1 ratio of healthy safflower is around 0.6, but in the rust infected plants it decreases gradually to below 0.2 at the later stages of infection (Daly *et al.*, 1957). This suggests that a striking replacement of respiratory pattern from the glycolytic pathway to the H.M.P. pathway may be evoked in the safflower hypocotyl by the rust infection. However, there is a possibility that such a change in the respiratory pattern could be due to an increase in the amount of the mycelium of the parasite, respiring through the H.M.P. pathway. Interestingly enough, DNP does not reduce the C-6:C-1 ratio of the uninfected tissue. Also, the DNP induced respiration is NaF sensitive. Since glycolysis is selectively inhibited by NaF, the nature of the pathogenically increased respiration is substantially

different from that of the DNP induced one. The experimental results of the Canadian workers are more or less the same. The C-6:C-1 ratio of the rusted wheat dropped to around 0.1–0.3 as compared to 0.5–0.6 of the uninfected wheat leaves (Shaw and Samborski, 1957). As shown in Fig. 6, they have clearly established the positive relationship between the magnitude of respiratory increase and the percentage participation of the H.M.P. pathway in rusted wheat and have shown an increase of the C-6:C-1 ratio in both the uninfected and the infected plant tissues

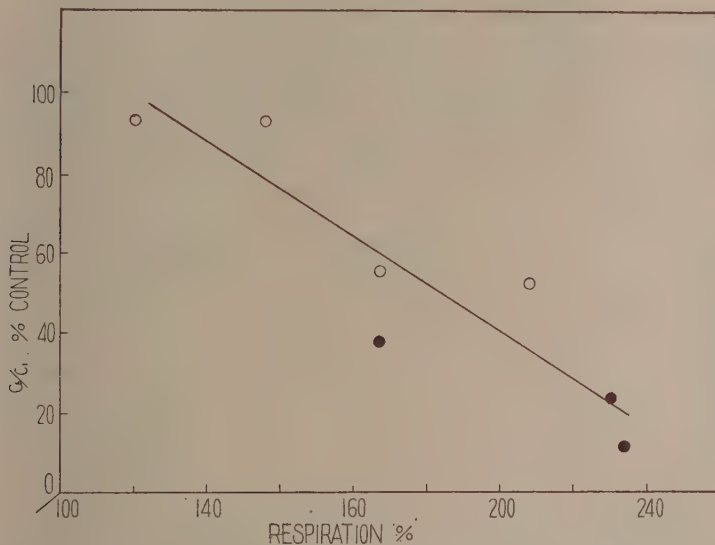


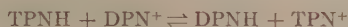
FIG. 6. The correlation between the C-6:C-1 ratio and oxygen uptake for rust-infected first leaves of Little Club and Khapli. Ordinate: C-6:C-1 ratio as a percentage of the value for uninfected controls. Abscissa: oxygen uptake as a percentage of the value for uninfected controls. Symbols: ● rusted Little Club ○ rusted Khapli. (After Shaw and Samborski (1957). Reproduced from Canadian Journal of Botany).

due to DNP addition. Applying the above described equations, different pictures of the contribution of the glycolytic or H.M.P. pathway in the rusted safflower and wheat can be obtained, but in every case they substantiate the role of the H.M.P. pathway in the respiration of infected plants. These observations have not only explained the marked output of  $\text{CO}_2$  by aerobic glucose breakdown, but also may give a new approach for the elucidation of carbohydrate metabolism and the respiratory pattern of infected plants.

We would now like to discuss some physiological aspects of the H.M.P. pathway in the metabolism of infected plants. It has already been observed that the pattern of carbohydrate metabolism is altered as the plant ages. That is, in the younger stages of plant growth, the glycolytic pathway is predominant, but as the plants grow older and differentiate, the H.M.P. pathway tends to replace it (Gibbs and Beevers, 1955). Also, it has been observed that with the addition of plant growth hormones, indoleacetic acid, and 2,4-dichlorophenoxyacetic acid, the C-6:C-1 ratio decreases, thus indicating the hormonal control of respiratory pattern (Humphreys and Dugger, 1957; Shaw, 1957). The above described augmented activity of the H.M.P. pathway in infected plants is another case of alteration of carbohydrate metabolism in plants. As has been previously mentioned, living organisms are able to adjust their metabolic pattern to a change of environment. Presumably, mutual intervention of main and supplementary pathways in regulating metabolism is a rather common feature in living organisms. Both the glycolytic and the H.M.P. pathways appear to be concurrently taking part in carbohydrate breakdown and are regulating the metabolism according to the energy requirement of cells. If the requirement becomes larger, functioning of the H.M.P. pathway might increase. It is not difficult to envision vigorous energy consumption in infected plant tissues accelerating the operation of the H. M. P. pathway, though the mechanism for inducing this is not certain. We suspect that the capacity of the glycolytic pathway may not be sufficient to fulfill the total energy requirement of the host. It has been proposed that the inhibition of either phosphofructokinase or hexokinase by oxidative systems may suppress the operation of the glycolytic pathway (Engelhardt and Sakov, 1943; Aisenberg *et al.*, 1957; Aisenberg and Potter, 1957). Furthermore, 6-phosphogluconate inhibits phosphoglucomutase. This fact may also explain the regulation of two alternative pathways (Parr, 1956). None of these possible implications can explain the reason for the more predominant participation of the H.M.P. pathway in infected plants, and in order to comprehend more fully the precise mechanism involved in the altered carbohydrate metabolism, the level of TPN (both oxidized and reduced forms), glucose-6-phosphate, and the net synthesis of the respective enzymes participating in the H.M.P. pathway in the cells should be investigated.

Thus far no experiments have been reported which deal with the interaction between the H.M.P. pathway and the respiratory chain systems, but this is also an important research subject necessary for the elucidation of the mechanism of respiratory increase. Transhydrogenase may be the most important enzyme in electron transfer systems from

TPNH to oxygen. This enzyme takes part in the transfer of hydrogen between DPN and TPN according to the following equation. Evidence of whether or not the oxidation of TPNH generated via the H.M.P. pathway



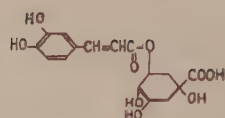
is coupled to oxidative phosphorylation is urgently needed, and there is a suggestion that TPNH is coupled to phosphorylation via the above transhydrogenase (Kaplan *et al.*, 1956).

Aromatic compounds often accumulated in infected plants are possibly synthesized via the H.M.P. pathway (see the next section). Also it has been shown that ribose-5-phosphate is synthesized by this pathway. Since ribose-5-phosphate is a constituent of ribonucleic acid, it would be of value to study the relationship between the H.M.P. pathway and the nucleic acid metabolism of virus-infected plant tissue (see Fig. 5).

#### IV. BIOCHEMICAL CHANGES ACCOMPANYING THE RESPIRATORY INCREASE

As has been briefly mentioned in Section II, D, abnormal metabolites such as coumarin and polyphenols are frequently synthesized in diseased plants (Fig. 7). Formation of polyphenols is a common feature occurring concomitantly with the respiratory increase; and coumarins have sometimes been observed to accumulate in infected plants. Studies on the mechanism of biogenesis of these aromatic substances have made considerable progress during the last several years. This is mainly due to the advancement of research of the biosynthesis of the aromatic amino acid—phenylalanine—by the use of genetic mutants of microorganisms and radioisotopic techniques (Davis, 1955). There are still some hypothetical points in the biosynthesis of phenylalanine and all of the intermediates have not been isolated and identified; however, erythrose-4-phosphate and phosphoenolpyruvic acid have been found to be the precursors which form the aromatic rings (Srinivasan *et al.*, 1955). Participation of sedoheptulose-7-phosphate as a direct precursor of shikimic acid has been disproved; instead, it acts as a precursor of erythrose-4-phosphate. Thus, it has been shown that sedoheptulose-7-phosphate is efficiently incorporated into phenylalanine. The erythrose-4-phosphate is obviously formed from sedoheptulose-7-phosphate through the H.M.P. pathway by the action of transaldolase. If other aromatic compounds are synthesized via routes similar to that of phenylalanine (see Fig. 5), perhaps the important function of the H.M.P. pathway is in the biosynthesis of various types of aromatic compounds. A predominant participation of the H.M.P. pathway in infected plants may thus have a close

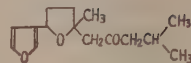




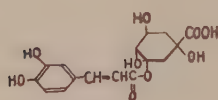
chlorogenic acid (a, b)



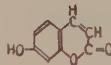
coumarin (d)



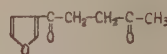
ipomeamarone (i, j)



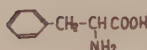
isochlorogenic acid (a)



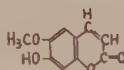
umbelliferon (a)



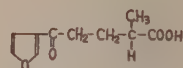
ipomeanine (j)



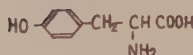
phenylalanine (c)



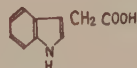
scopoletin (a, e, f)



batatic acid (j)



tyrosine (c)



indoleacetic acid (g, h)

FIG. 7. Some metabolites accumulated in infected plants.

	Host	Pathogen	Reference
a	Sweet potato	<i>Ceratostomella fimbriata</i>	Uritani and Akazawa (1955b)
b	Potato	<i>Helminthosporium carbonum</i>	Kuč <i>et al.</i> (1956)
c	Wheat	<i>Puccinia graminis</i>	Shaw (1957)
d	Rice plant	<i>Piricularia oryzae</i>	Tamari (1957)
e	Tobacco	Tomato spotted wilt virus	Best (1948)
f	Potato	<i>Phytophthora infestans</i>	Fuchs (1956)
g	Wheat	<i>Puccinia graminis</i>	Shaw (1957)
h	Safflower	<i>Puccinia carthami</i>	Daly and Inman (1958)
i	Sweet potato	<i>Ceratostomella fimbriata</i>	Hiura (1941)
j	Sweet potato	<i>Ceratostomella fimbriata</i>	Kubota and Matsuura (1953)

connection with the biosynthesis of polyphenols and coumarin derivatives (Uritani and Akazawa, 1955b).

The effect of rust infection on the amino acid metabolism of wheat leaves has been studied by two groups (Shaw, 1957; Rohringer, 1957).

Shaw and Colotelo have shown that in the susceptible species of wheat infected with stem rust several amino acids, including aromatic amino acids, have increased notably as compared to the levels in the uninfected plant. The amino acid composition of protein hydrolyzates of the rusted wheat has also greatly changed, and they suggested that an alteration in the enzyme systems concerned with the metabolism of those compounds may occur under pathological conditions.

There are several reports indicating an increase in the auxin concentration of the infected plant, though it is very low indeed in a healthy one (Pilet, 1952; Shaw, 1957; Shaw and Hawkins, 1958; Daly and Inman, 1958). All of them have noticed hypertrophy or cell elongation of the host when infected and have discussed this phenomenon with respect to the influence of auxin. Shaw's group (Shaw, 1957; Shaw and Hawkins, 1958) analyzed the amount of indoleacetic acid in a quantitative manner and simultaneously looked into the enzymatic oxidation of radioactive indoleacetic acid ( $C^{14}OOH$ ) by analyzing the release of  $C^{14}O_2$ . The concentration of indoleacetic acid in the infected leaves of the susceptible species (Little Club) of wheat decreased initially after infection but 10 days after infection was ten to twenty times higher than that of the uninfected leaves. A reciprocal relationship was observed in the activity of indoleacetic acid oxidase. However, no such increase of indoleacetic acid was seen in the case of infected wheat leaves of the resistant species (Khapli). This is, perhaps, due to a constant higher activity of indoleacetic acid oxidase in the infected plant of the resistant species. The American group analyzed the auxin content of safflower hypocotyl infected with *Puccinia carthami* during the course of infection and obtained results similar to those of the above authors. Shaw and his co-workers (1958) have observed that indoleacetic acid lowers the C-6:C-1 ratio. From this fact together with the lowered C-6:C-1 ratio in rusted and mildewed leaves, it seems probable that the auxin is conceivably somehow controlling the respiratory metabolism of the host tissue. However, firm conclusions should not be drawn from the following observations: (1) lower C-6:C-1 due to indoleacetic acid addition is attributable to the lower recovery of C-6 whereas it is due to the higher recovery of C-1 in the rusted wheat leaves; (2) also, as has been described above, there was an apparent decrease of indoleacetic acid content in the resistant species of wheat leaves, irrespective of the lower C-6:C-1 ratio (Shaw, 1957; Shaw *et al.*, 1958).

Three compounds have been isolated from the sweet potato infected by the black rot fungus (i.e., ipomeamarone, ipomeanin, and batatic acid) and their chemical structures (see Fig. 7) proven (Hiura, 1941; Kubota

and Matsuura, 1953). As the chemical structure of ipomeamarone shows, this is a kind of sesquiterpenoid with a molecular formula of a reduced type. We feel that these compounds may be synthesized by an unknown alternative pathway of the host metabolism (Uritani and Akazawa, 1955b). Metabolism of  $C_2$  compounds, which are normally oxidized by the TCA cycle or sometimes utilized to synthesize fatty acid or phospholipid, may be bypassed to form isoprenoid units and eventually lead to a synthesis of ipomeamarone and its derivatives.

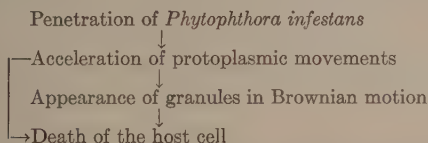
#### V. ALTERATION OF THE RESPIRATION IN RELATION TO DEFENSE MECHANISMS OF THE HOST

Sufficient data are not available to formulate a straightforward hypothesis on the relationship between the altered respiratory pattern in infected plant tissue and defense reactions, but some speculation here may guide future study. Conceivably, metabolic patterns evolved in plants allowing them to adjust to a changing environment. Probably, defense devices of plants have been improved from a very primitive to a highly advanced type. As stressed repeatedly, the increase in respiratory rate is a comprehensive phenomenon observed in many plants exposed to varied stimuli ranging from a simple mechanical treatment to a complex pathogenic infection. Therefore, it is important to realize that respiration of the host tissue increases in response to an environmental change. This applies also to the defense mechanisms of plant tissue against pathogens as well as other injurious agents.

From a large amount of experimental data, it appears that respiratory increase and accompanying metabolism are more or less parallel to the magnitude of the pathogenic penetration of the parasite in the host. The more severe the pathogenic infection becomes, the more intensive is the metabolic activity. Thus, an augmented respiratory rate is an expression of the host response to the penetrating pathogen. However, this does not mean that the magnitude of the respiratory increase is a measure of the resistance activity of host tissue. In the infection of rust resistant wheat, the data of Shaw's group have shown a rapid respiratory increase followed by a sharp drop of respiratory rate as compared to the respiratory rate in the susceptible species. The magnitude of increased respiration was not, however, greater than that observed in the susceptible species (Fig. 4). Such features have been observed by several workers (Millerd and Scott, 1956; Király and Farkas, 1956). In the case of the potato-*Phytophthora* complex studied by Tomiyama and his co-workers (1955, 1956), the change in the respiratory rate of the resistant variety is more pronounced than that of the susceptible variety both in the sharp rise and in the total amount of oxygen uptake. Observations

of Uritani and Akazawa (1955b) on the black rot disease of sweet potato indicate a more rapid increase and a greater magnitude of the respiratory rate in the resistant than in the susceptible varieties (Table II). From these observations, it appears that the initial sharp rise in the respiratory rate of infected plants may be connected with the defense mechanism of the plants.

In addition, several people have found that they can efficiently diminish the resistant activity of the host by inhibiting the phosphorylation reaction. In other words, the pathogen is able to penetrate the host more readily following a treatment of this kind. For instance, if a sweet potato infected with *Ceratostomella fimbriata* is left under conditions of high humidity and low oxygen tension, no marked enhancement of metabolism of the host is observed; rather, the fermentation process predominates in the host tissue. Under these conditions, the pathogen spreads over the surface of the sweet potato and penetrates readily. Several experimental results have shown that treatment of potato tubers with ethanol facilitates greatly the penetration of *Phytophthora infestans* (Meyer, 1939; Behr, 1949). According to Tomiyama's experiment, processes such as respiratory increase, syntheses of water-soluble protein and polyphenols are suppressed considerably in the presence of ethanol; accompanying this is a decrease in the resistant activity of the host (Tomiyama *et al.*, 1956, 1957). Similar observation has been reported for rice plants infected with *Piricularia oryzae* (Tochinai, 1951). Walker and his co-workers have shown that tomato plants treated with some respiratory inhibitors, including the uncoupling agent DNP, lose their resistant activity to *Fusarium oxysporum* f. *lycopersici*. They have suggested that some very labile substance, which is closely connected to the phosphate metabolism of the host, might be an important factor in the resistant activity of tomato (Gothoskar *et al.*, 1955; Walker and Stahmann, 1955). According to Tomiyama's cytological study (Tomiyama, 1954, 1955a, b), midrib cells of potato have shown the following sequential microscopical features when infected with *Phytophthora infestans*. In most cases, immediately after the active protoplasmic streaming, hypersensitive death of cells follows. However, if the tissue is treated with DNP, the death of



the host is remarkably delayed and it becomes susceptible to the parasite (Tomiyama, 1957). As these several experimental results show, there



definitely exist defense mechanisms of the host which demand respiratory energy that is possibly linked to the phosphorylation system.

Accompanying the respiratory increase, many compounds including polyphenols and coumarins are synthesized and some of them may be functioning as antipathogenic principles of the host. Although the production of antiparasitic principles by the host will be discussed in Chapter 12 of this volume, a brief description is given here. Kué and his associates (1956) have studied the accumulation of polyphenols in potato tubers infected by *Helminthosporium carbonum* and suggested the role of the substances in natural immunity of plants. In the case of the black rot of sweet potato, 1-5 mg. polyphenols per gram fresh weight tissues accumulated. However, with these concentrations, there is little inhibition of the black rot fungus. Apparently there is no positive relationship between the invasion of the pathogen and the amount of polyphenols synthesized (Table II) in the host (Uritani and Akazawa, 1955b). Studies of the antipathogenic nature of polyphenols in infected plants and the role of polyphenol oxidase in relation to the physiological action of polyphenols are (from a biochemical viewpoint) most pertinent in the field of phytopathology. Coumarin substances were isolated from the diseased plants and some of them exhibited fungitoxic action (Best, 1948; Uritani and Hoshiya, 1953; Fuchs, 1956; Tamari, 1957). Ipomeamaron, found in the injured part of the diseased sweet potato is definitely an anti-parasitic substance (Table II) and the amounts are roughly parallel to the degree of resistance of the plant (Uritani and Akazawa, 1955a, b).

When a resistant variety of plant is attacked by a pathogen, instant necrosis (denaturation, coagulation of proteins, and browning) precedes the penetration of the pathogen into healthy tissue. This phenomenon is called hypersensitivity (see Chapter 13 of this volume). Probably, hypersensitivity plays an important role in the defense mechanism of the host against the pathogen by producing antipathogenic agents and forming protecting barriers. Müller (1956) isolated some substances from the inner epidermis of pea pods infected by *Sclerotinia fructicola* and *Phytophthora infestans* which have an inhibitory action against the pathogen and also induce necrosis of the host. Rubin feels that the activation of polyphenol oxidase in potato tissue infected with *Phytophthora infestans* is the crucial factor in inducing necrosis (Rubin and Axenova, 1957), and the excellent work of the German researchers is considered to coincide with this opinion (Fuchs and Kotte, 1954; Christiansen-Weniger, 1955). These workers found that by treating the potato tuber with some respiratory inhibitors, particularly by copper chelating agents, the host became susceptible to *Phytophthora infestans*. Japanese workers have

emphasized the fact that hypersensitivity accompanying the respiratory increase is an important factor in the resistant action of the host (Tomiya, 1954, 1955a, b, 1957; Hirai, 1955; Suzuki *et al.*, 1957). Hypersensitivity is the result of the interaction between host and pathogen. Although the mechanism of the phenomenon has not as yet been clarified, we might postulate the following: when attacked by the pathogen, the metabolism of the host cell is activated. Some enzymes and/or metabolites produced by the pathogen damage the protoplasmic function of the cell. When the cell is injured some substances produced by it stimulate necrosis, thus leading to hypersensitivity.

Yet, the fact that respiratory increase does not always accompany hypersensitivity should be emphasized. For instance, when a nonresistant sweet potato variety is attacked by *Ceratostomella fimbriata*, the above described features of metabolic alteration are observed and ipomeamaron is synthesized in the injured area. The pathogen continues to penetrate gradually into the host tissue, yet profound hypersensitivity is not observed. We may postulate the production of hypersensitivity inducers by pathogen in host cells and formation of hypersensitivity stimulators by host cells. In the case of sweet potato, nonresistant to the black rot fungus, the potency of these two factors might not be strong enough to cause necrosis preceding penetration of the pathogen. Those factors are hypothetical ones and Müller's phytoalexins, Rubin's activated polyphenolase-polyphenol system, and ipomeamarone and other abnormal metabolites in the infected sweet potato might be considered to be examples of hypersensitive stimulators (Uritani and Akazawa, 1955b; Suzuki *et al.*, 1957). Study on the function of necrosin discovered in the inflammation of infected animal tissue, would be of value in elucidating the function of this proposed factor (Menkin, 1950).

Browning is a characteristic feature of hypersensitivity in the host, but the exact mechanism by which this biochemical reaction takes place is not known. Histological observations of necrosis in the injured tissue of infected plants lead us to the following assumption: first, polyphenolic compounds are oxidized by polyphenol oxidase which may exist in a latent state in the intact plant tissue and be activated on exposure to pathogenic infection. Second, the oxidized polyphenols, now quinones, are condensed to form polyquinoid structures or sometimes react with amino acids or proteins to form melanin substances. The net effect of these reactions may constitute a defense mechanism of the host by forming a barrier.

In conclusion, respiratory increase of host tissue appears to be a prerequisite for the induction of the resistant action of the plant. Some other biochemical events occurring concurrently with the respiratory

alteration may amplify the resistance of the host through their antipathogenic action. And finally, hypersensitive reactions of the host preceding the pathogenic infection are necessary to establish the resistance action. Those over-all relationships are diagrammatically exemplified in Fig. 8, in which the change of respiration in the infected sweet potato and its resistance against *Ceratostomella fimbriata* are shown.

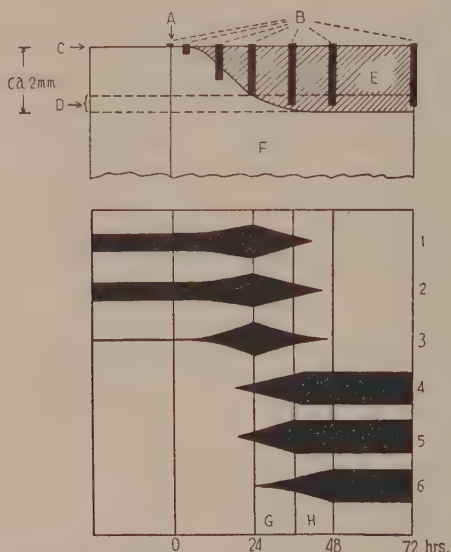


FIG. 8. The relationship between change of respiration in the infected sweet potato and its resistance against *Ceratostomella fimbriata*. The upper part of the diagram shows development of fungus penetration (B) concomitant with the progress of injured tissue (E) and the gradual decrease of healthy tissue (F). At 48 hr. after inoculation (A) of the fungus on the surface (C) of sweet potato slice, the infection is apparently prevented by the resistance action of the host and further penetration is not observed. The lower part shows the quantitative change of some biochemical events (1-6) observed in (D) (the tissue 1.75 mm. inside from the surface (C)) as a consequence of the pathogenic infection. (1) Change of over-all ATP-utilizing system. (2) Change in the respiratory rate. (3) Change of the amount of coumarins and polyphenols. (4) Inactivation and denaturation of protein. (5) Synthesis of ipomeamarone. (6) Browning. Up to 24 hr. after the inoculation, the (D) part is healthy and not infected, but afterwards this part is gradually injured until about 48 hr., and is playing a role in the resistance during 24 to 48 hr. Presumably this stage is divided into two parts (G) and (H). At (G) stage, the resistance might be attributable to some antibiotic factors such as coumarins (3), polyphenols (3), and ipomeamarone (5). At (H) stage the resistance might be mainly due to the barrier formation in which events of (4) and (6) are involved.

## ACKNOWLEDGMENTS

We are deeply indebted to Dr. R. D. Durbin, University of California, Berkeley,\* for his invaluable advice during the preparation of the manuscript and for his many helpful criticisms. We also express our thanks to Dr. M. Shaw, University of Saskatchewan, Canada, and to Drs. Z. Király and G. L. Farkas, Research Institute for Plant Protection, Budapest, Hungary, for their valuable suggestions and for allowing us to see their manuscripts before their publication. Gratitude is also expressed to Professor T. Hirai, Nagoya University, for very valuable discussions with him. A portion of the manuscript was prepared when one of us (T. A.) was at the Department of Agricultural Biochemistry, University of California, Berkeley, and he wishes to thank colleagues there for their kind discussion on the material covered and also to express appreciation for the use of the University Library through which the work was greatly advanced.

## REFERENCES

- Aisenberg, A. C., and Van R. Potter. 1957. Studies on the Pasteur-effect. II. Specific mechanisms. *J. Biol. Chem.* **224**: 1115-1127.
- Aisenberg, A. C., B. Reinfarje, and Van R. Potter. 1957. Studies on the Pasteur-effect. I. General observations. *J. Biol. Chem.* **224**: 1099-1113.
- Akazawa, T. 1956. Metabolic activation of white potato tissue infected with *Ceratostomella fimbriata*. *J. Biochem. (Tokyo)* **43**: 589-593.
- Akazawa, T., and I. Uritani. 1955. Respiratory increase and phosphorus and nitrogen metabolism in sweet potato infected with black rot. *Nature* **176**: 1071-1072.
- Allen, P. J. 1942. Changes in the metabolism of wheat leaves induced by infection with powdery mildew. *Am. J. Botany* **29**: 425-435.
- Allen, P. J. 1953. Toxins and tissue respiration. *Phytopathology*. **43**: 221-229.
- Allen, P. J. 1954. Physiological aspects of fungus diseases of plants. *Ann. Rev. Plant Physiol.* **5**: 225-248.
- Arzichowskaja, E. V. 1946. On the physiology of host-parasite relations of the *Botrytis cinerea*-cabbage complex. (Orig. in Russian.) *Mikrobiologiya* **15**: 47-56; *Chem. Abstr.* **43**: 1840.
- Asada, Y. 1957. Studies on the susceptibility of Akiuchi-rice plant to *Helminthosporium* blight. IV. Changes of nitrogen compounds, carbohydrates, reducing ascorbic acid and respiration accompanied by the infection of *Cochliobolus miyabeanus* and existence of hyphae in diseased spots. *Ann. Phytopathol. Soc. Japan* **22**: 103-106.
- Axelrod, B., and H. Beevers. 1956. Mechanism of carbohydrate breakdown. *Ann. Rev. Plant Physiol.* **7**: 267-298.
- Beevers, H. 1953. 2,4-Dinitrophenol and plant respiration. *Am. J. Botany* **40**: 91-96.
- Beevers, H. 1954. The oxidation of reduced diphosphopyridine nucleotide by an ascorbate system from cucumber. *Plant Physiol.* **29**: 265-269.
- Beevers, H., and M. Gibbs. 1954a. The direct oxidation pathway in plant respiration. *Plant Physiol.* **29**: 322-324.
- Beevers, H. and M. Gibbs. 1954b. Participation of the oxidative pathway in yeast respiration. *Nature* **173**: 640.
- Behr, L. 1949. Über den Einfluss von narkotisch wirkenden Stoffen auf die Wundperidermbildung und Resistenz der Kartoffelknolle gegenüber *Phytophthora*

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- infestans* de By. und Vertretern der Gattung *Fusarium* Lk. *Phytopathol. Z.* **15**: 407-446.
- Best, R. J. 1948. Studies on a fluorescent substance present in plants. Part 3. The distribution of scoporetin in tobacco plants and some hypotheses on its part in metabolism. *Australian J. Exptl. Biol. Med. Sci.* **26**: 223-230.
- Bloom, B., and D. Stetten, Jr. 1955. The fraction of glucose catabolism via the glycolytic pathway. *J. Biol. Chem.* **212**: 555-563.
- Bonner, W. D., Jr. 1957. Soluble oxidases and their function. *Ann. Rev. Plant. Physiol.* **8**: 427-452.
- Chance, B., and C. R. Williams. 1956. The respiratory chain and oxidative phosphorylation. *Advances in Enzymol.* **17**: 65-134.
- Christiansen-Weniger, E. 1955. Versuche zur stoffwechselphysiologischen Beeinflussung der Reaktion der Kartoffelknolle auf *Phytophthora infestans* de By. *Phytopathol. Z.* **25**: 153-180.
- Daly, J. M., and R. E. Inman. 1958. Changes in auxin levels in safflower hypocotyls infected with *Puccinia carthami*. *Phytopathology* **48**: 91-97.
- Daly, J. M., and R. M. Sayre. 1957. Relation between growth and respiratory metabolism in safflower infected with *Puccinia carthami*. *Phytopathology* **47**: 163-168.
- Daly, J. M., R. M. Sayre, and J. H. Pazur. 1957. The hexose monophosphate shunt as the major respiratory pathway during sporulation of rust of safflower. *Plant Physiol.* **32**: 44-48.
- Davis, B. D. 1955. Biosyntheses of the aromatic amino acids. In "A Symposium on Amino Acid Metabolism" (W. D. McElroy and B. Glass, eds.). Johns Hopkins Press, Baltimore, Maryland. pp. 799-811.
- Dickens, F. 1951. Anaerobic glycolysis, respiration and the Pasteur-effect. In "The Enzymes" (J. B. Sumner and K. Myrback, eds.), Vol. 2, Part I. Academic Press, New York. pp. 624-672.
- Dimond, A. E., and P. E. Waggoner. 1953. On the role of vivotoxins in plant disease. *Phytopathology* **43**: 229-235.
- Eichenberger, E., and K. V. Thimann. 1957. Terminal oxidases and growth in plant tissues. IV. On the terminal oxidases of etiolated pea internodes. *Arch. Biochem. Biophys.* **67**: 466-478.
- Engelhardt, V. A., and N. E. Sakov. 1943. The mechanism of the Pasteur-effect. *Biokhimiya* **8**: 9-36.
- Farkas, G. L. 1957. Some notes on the metabolic interactions between host and parasite. *Acta Biol. Acad. Sci. Hung.* **7**: 311-323.
- Farkas, G. L., and Z. Király. 1955. Studies on the respiration of wheat infected with stem rust and powdery mildew. *Physiol. Plantarum.* **8**: 877-887.
- Farkas, G. L., and Z. Király. 1958. Enzymological aspects of plant diseases. I. Oxidative Enzymes. *Phytopathol. Z.* **31**: 251-272.
- Fuchs, W. H. 1956. Ein Beitrag zur pathologischen Physiologie. *Angew. Botan.* **30**: 141-146.
- Fuchs, W. H., and E. Kotte. 1954. Zur Kenntnis der Resistenz von *Solanum tuberosum* gegen *Phytophthora infestans* de By. *Naturwiss.* **41**: 169-70.
- Gäumann, E., and O. Jaag. 1947. Die physiologischen Grundlagen des parasitogenen Welkens. *Ber. schweiz. botan. Ges.* **57**: 3-34.
- Gibbs, M., and H. Beevers. 1955. Glucose dissimilation in the higher plant. Effect of age of tissue. *Plant Physiol.* **30**: 343-347.

- Gothoskar, S. S., R. P. Scheffer, M. A. Stahmann, and J. C. Walker. 1955. Further studies on the nature of *Fusarium*-resistance in tomato. *Phytopathology* **45**: 303-307.
- Gottlieb, D., and J. M. Garner. 1946. Rust and phosphorus distribution in wheat leaves. *Phytopathology* **36**: 557-564.
- Greenham, C. G., and K. O. Müller. 1956. Conductance changes and responses in potato tubers following infection with various strains of *Phytophthora* and with *Pythium*. *Australian J. Biol. Sci.* **9**: 199-212.
- Heitefuss, R. 1957. Untersuchungen zur pathologischen Physiologie von *Peronospora parasitica* auf *Brassica oleracea*. Ph. D. Thesis, pp. 1-107.
- Hellings, J. J. A. 1942. Über den Einfluss von Substanzen, die von Pilzen gebildet werden, auf die Atmung des Kartoffelknollengewebes. *Rec. trav. botan. néerl.* **38**: 151-286.
- Hill, R., and E. F. Hartree. 1953. Hematin compounds in plants. *Ann. Rev. Plant Physiol.* **4**: 115-150.
- Hirai, T. 1955. The mechanism of resistance of plants against pathogens. Ph.D. Thesis. pp. 1-78.
- Hirai, T., and N. Suzuki. 1956. Mechanism of resistance in higher plants. *Nôgyô Gijutsu* **11**: 404-408.
- Hiura, M. 1941. Studies in storage and rot of sweet potato (2). *Gifu Nôriu Semmon Gokkô Gakurujutsu Hôkoku* **50**: 1-5.
- Hulme, A. C. 1955. The climacteric rise in respiration in relation to changes in the equilibrium between protein synthesis and breakdown. *J. Exptl. Botany* **5**: 159-172.
- Humphreys, T. E., and W. M. Dugger, Jr. 1957. The effect of 2,4-dichlorophenoxy acetic acid on pathway of glucose catabolism in higher plants. *Plant Physiol.* **32**: 136-140.
- James, W. O. 1953a. "Plant Respiration." Oxford Univ. Press, London and New York. 282 pp.
- James, W. O. 1953b. The use of respiratory inhibitors. *Ann. Rev. Plant Physiol.* **4**: 59-90.
- James, W. O., and D. Boutler. 1955. Further studies on the terminal oxidases in the embryos and growing roots of barley. *New Phytologist* **54**: 1-12.
- Johnson, M. J. 1941. The role of aerobic phosphorylation in the Pasteur-effect. *Science* **94**: 200-202.
- Kaplan, N. O., M. N. Schwartz, M. E. French, and M. M. Giotti. 1956. Phosphorylative and nonphosphorylative pathways of electron transfer in rat liver mitochondria. *Proc. Natl. Acad. Sci. U. S. A.* **42**: 481-487.
- Kern, H. 1956. Problems of incubation in plant diseases. *Ann. Rev. Microbiol.* **10**: 351-368.
- Király, Z., and G. L. Farkas. 1956. Enzymology of diseased plants and the problem of resistance. *Kiserletügyi Közlemények* **50**: 149-166.
- Király, Z., and G. L. Farkas. 1957. On the role of ascorbic oxidase in the parasitically increased respiration of wheat. *Arch. Biochem. Biophys.* **66**: 474-485.
- Korkes, S. 1956. Carbohydrate metabolism. *Ann. Rev. Biochem.* **25**: 685-734.
- Krebs, H. A., and H. L. Kornberg. 1957. "Energy Transformation in Living Matter." Springer, Berlin. 298 pp.
- Kubota, T., and T. Matsuura. 1953. Chemical studies on the black rot disease of sweet potato. *J. Chem. Soc. Japan.* **74**: 101-109, 197-199, 248-251, 668-670.

- Kuč, J., R. E. Henze, A. J. Ullstrup, and F. W. Quackenbush. 1956. Chlorogenic and caffeic acids as fungitoxic agents produced by potatoes in response to inoculation with *Helminthosporium carbonium*. *J. Am. Chem. Soc.* **78**: 3123-3125.
- Lakshmanan, M., and C. S. Venkata Ram. 1957. Influence of *Fusarium* culture filtrates on respiratory changes in cotton. *Proc. Indian Acad. Sci. Section B.* **46**: 131-137.
- Lardy, H. A. 1952. The role of phosphate in metabolic control mechanisms. In "The Biology of Phosphorus" (L. F. Wolterink, ed.), Michigan State Univ. Press, East Lansing, Michigan. pp. 131-147.
- Lardy, H. A., and H. Wellman. 1952. Oxidative phosphorylations: role of inorganic phosphate and acceptor systems in control of metabolic rates. *J. Biol. Chem.* **195**: 215-224.
- Laties, G. G. 1957. Respiration and cellular work and the regulation of the respiration rate in plants. In "Survey of Biological Progress" (B. Glass, ed.), Vol. III. Academic Press, New York. pp. 215-299.
- Link, G. K. K., and R. M. Klein. 1951. Studies on the metabolism of plant neoplasms. II. The terminal oxidase patterns of crown gall and auxin tumors of tomato. *Botan. Gaz.* **113**: 190-195.
- Loughman, B. C., and R. P. Martin. 1957. Methods and equipment for the study of the incorporation of phosphorus by intact barley plants in experiments of short duration. *J. Exptl. Botany* **8**: 272-279.
- Loughman, B. C., and R. S. Russel. 1957. The absorption and utilization of phosphate by growing barley plants. IV. The initial stage of phosphate metabolism in roots. *J. Exptl. Botany* **8**: 280-293.
- Lynen, F., and R. Königsberger. 1951. Zum Mechanismus der Pasteurschen Reaktion. Der Phosphat-Kreislauf in der Hefe und seine Beeinflussung durch 2,4-Dinitrophenol. *Ann.* **573**: 60-84.
- Menkin, V. 1950. "Newer Concepts of Inflammation." C. C Thomas, Springfield, Illinois. 145 pp.
- Meyer, G. 1939. Zellphysiologische und anatomische Untersuchungen über die Reaktion der Kartoffelknolle auf den Angriff der *Phytophthora infestans* bei Sorten verschiedener Resistenz. *Arb. biol. Reichsanstalt Land- u. Forstwirtschaft. Berlin Dahlem* **23**: 97-132.
- Millard, A., and K. Scott. 1955. A phytopathogenic toxin formed in barley infected with powdery mildew. *Australian J. Sci.* **18**: 63-64.
- Millard, A., and K. Scott. 1956. Host pathogen relations in powdery mildew of barley. II. Changes in respiratory pattern. *Australian J. Biol. Sci.* **9**: 37-44.
- Millard, A., J. Bonner, and J. B. Biale. 1955. The climacteric rise in fruit respiration as controlled by phosphorylative coupling. *Plant Physiol.* **28**: 521-31.
- Müller, K. O. 1956. Einige einfache Versuche zum Nachweis von Phytoalexinen. *Phytopathol. Z.* **27**: 237-254.
- Naef-Roth, S., and P. Reusser. 1954. Über die Wirkung der Fusarinsäure auf den Gaswechsel von Tomaten-Blattgewebe. *Phytopathol. Z.* **22**: 281-287.
- Owen, P. C. 1955. The respiration of tobacco leaves in the 20-hour period following inoculation with tobacco mosaic virus. *Ann. Appl. Biol.* **43**: 114-121.
- Owen, P. C. 1957. The Effect of infection with tobacco etch virus on the rates of respiration and photosynthesis of tobacco leaves. *Ann. Appl. Biol.* **45**: 327-331.
- Pappenheimer, A. M. 1954. Some effects of bacteria and their products on host-cell

- metabolism. In "Cellular Metabolism and Infections" (E. Racker, ed.). Academic Press, New York, pp. 102-116.
- Paquin, R., and E. R. Waygood. 1957. The effect of *Fusarium* toxins on the enzymatic activity of tomato hypocotyl mitochondria. *Can. J. Botany* **35**: 207-218.
- Parr, C. W. 1956. Inhibition of phosphoglucomutase. *Nature* **178**: 1401.
- Pilet, P. E. 1952. Problème hormonal concernant l'*Endophyllum sempervivi* Lev. parasite du *Sempervivum tectorum* L. *Ber. schweiz. botan. Ges.* **62**: 269-274.
- Pozsár, B. I., and Z. Király. 1958. Effect of rust infection on oxidative phosphorylation of wheat leaves. Personal communication.
- Racker, E., ed. 1954. "Cellular Metabolism and Infections." Academic Press, New York. 196 pp.
- Robertson, R. N., and J. A. Pearson. 1954. The physiology of growth in apple fruits. VI. The control of respiration rate and synthesis. *Australian J. Biol. Sci.* **7**: 1-17.
- Rohringer, R. 1957. Untersuchungen zur Biochemie von Weizenkeimpflanzen nach Infektion mit *Puccinia graminis tritici*, Erikss. und Henn., ph. R. 126A. *Phytopathol. Z.* **29**: 45-64.
- Rowan, K. S., D. E. Seaman, and J. S. Turner. 1956. Phosphorylation as a possible factor in the Pasteur-effect in plants. *Nature* **177**: 833-834.
- Rubin, B. A., and V. A. Axenova. 1957. Participation of the polyphenolase system in the defense reactions of potato against *Phytophthora infestans*. (Orig. in Russian.) *Biokhimiya* **22**: 202-209.
- Rubin, B. A., and E. P. Chetverikova. 1955. On the role of oxidative processes in the resistance of cabbage to *Botrytis cinerea*. (Orig. in Russian.) *Biokhim. Plodov. i Ovoshchei Akad. Nauk. S. S. S. R. Inst. Biokhim. im. A. N. Bakha* **3**: 43-78; *Chem. Abstr.* **50**: 2754.
- Rubin, B. A., E. V. Arzichowskaja, and T. A. Proskurnikova. 1947. Oxidative changes of phenols and their relation to the resistance of potatoes against *Phytophthora infestans*. (Orig. in Russian.) *Biokhimiya*. **12**: 141-152; *Chem. Abstr.* **41**: 5175.
- Rubin, B. A., E. P. Chetverikova, and E. V. Arzichowskaja. 1955. Oxidative system and plant immunity. (Orig. in Russian.) *Zhur. Obshchei. Biol.* **16**: 106-118; *Chem. Abstr.* **50**: 6592.
- Samborski, D. J., and M. Shaw. 1956. The physiology of host-parasite relations. II. The effect of *Puccinia graminis tritici* Erikss. and Henn. on the respiration of the first leaf of resistant and susceptible species of wheat. *Can. J. Botany* **34**: 601-619.
- Scheffer, R. P., and J. C. Walker. 1954. The physiology of *Fusarium* wilt of tomato. *Phytopathology* **43**: 116-125.
- Sempio, C. 1950. Metabolic resistance to plant disease. *Phytopathology* **40**: 799-822.
- Shaw, M. 1957. Studies on the physiology of the host-parasite relations of wheat stem-rust. Proc. IVth International Congress on Crop Protection, Hamburg, Germany. September 1957. In press.
- Shaw, M., and A. R. Hawkins. 1958. The physiology of host-parasite relations. V. A preliminary examination of the level of free endogenous indoleacetic acid in rusted and mildewed cereal leaves and their ability to decarboxylate exogenously supplied radioactive indoleacetic acid. *Can. J. Botany* **36**: 1-16.
- Shaw, M., and D. J. Samborski. 1956. The physiology of host-parasite relations. I. The accumulation of radioactive substances at infections of facultative and obligate parasites including tobacco mosaic virus. *Can. J. Botany* **34**: 389-405.



- Shaw, M., and D. J. Samborski. 1957. The physiology of host-parasite relations. III. The pattern of respiration in rusted and mildewed cereal leaves. *Can. J. Botany* **35**: 389-407.
- Shaw, M., S. A. Brown, and D. Rudd-Jones. 1954. Uptake of radioactive carbon and phosphorus by parasitised leaves. *Nature* **173**: 768.
- Shaw, M., D. J. Samborski, and A. Oaks. 1958. Some effects of indoleacetic acid and maleic hydrazide on the respiration and flowering of wheat. *Can. J. Botany* **36**: 233-237.
- Siekevitz, P., and Van R. Potter. 1953. Intramitochondrial regulation of oxidative rate. *J. Biol. Chem.* **201**: 1-13.
- Srinivasan, P. R., M. Katagiri, and D. B. Sprinson. 1955. The enzymatic synthesis of shikimic acid from D-erythrose-4-phosphate and phosphoenolpyruvate. *J. Am. Chem. Soc.* **77**: 4943.
- Stroganov, B. P. 1947. Changes in the stem of cotton upon infection with wilt disease. (Orig. in Russian.) *Izvest. Akad. Nauk. S.S.S.R. Ser. Biolo. No. 6*: 777-789.
- Suzuki, N., K. Kasai, Y. Yamazaki, T. Araki, S. Toyodo, and T. Takahashi. 1957. Studies on the violet root rot of sweet potatoes. *Bull. Natl. Inst. Agr. Sci. (Japan). Ser. C. No. 8*: 1-173.
- Tamari, K. 1955. Biochemical studies on the rice plant infected with *Piricularia oryzae*. *Kagaku (Tokyo)* **25**: 18-23. (In Japanese.)
- Tamari, K. 1957. Biochemical studies on *Piricularia oryzae*. *Shokubutsu Boeki* **11**: 233-239. (In Japanese.)
- Tamari, K., and J. Kaji. 1953. Studies on the mechanism of injurious action of fusarinic acid, a metabolic product of the causative mold "Bakanae" disease of rice plant, on plant growth. *J. Agr. Chem. Soc. Japan* **27**: 245-249.
- Thatcher, F. S. 1942. Further studies of osmotic and permeability relations in parasitism. *Can. J. Research* **C20**: 283-311.
- Thatcher, F. S. 1943. Cellular changes in relation to rust resistance. *Can. J. Research* **C21**: 151-172.
- Tochinai, Y. 1951. The functional resistance in Plants. *Forsch. Gebiet Pflanzenkrankheiten* **4**: 1-7.
- Tombesi, L. 1949. Respiration and activity of oxidase and catalase during cicatrization in sound and infected tubers of potato (*Solunum tuberosum*). *Ann. sper. agrar. (Rome)* **3**: 1227-50; *Chem. Abstr.* **44**: 4966.
- Tomiyama, K. 1954. Cytological studies of resistance of potato plants to *Phytophthora infestans*. I. The process of alteration in the host cell produced by invasion of parasite. *Hokkaido Natl. Agr. Expt. Sta., Research Bull.* **67**: 28-38.
- Tomiyama, K. 1955a. Cytological studies on resistance of potato plants to *Phytophthora infestans*. II. The death of the intracellular hyphae in the hypersensitive cell. *Ann. Phytopathol. Soc. Japan* **19**: 149-154.
- Tomiyama, K. 1955b. Cytological studies on resistance of potato plants to *Phytophthora infestans*. III. The time required for the browning of midrib cell of potato plants infected by *Phytophthora infestans*. *Ann. Phytopathol. Soc. Japan* **19**: 165-169.
- Tomiyama, K. 1957. Cell-physiological studies on the resistance of potato plants to *Phytophthora infestans*. V. Effect of 2,4-dinitrophenol upon the hypersensitive reaction of potato plant cell to infection by *Phytophthora infestans*. *Ann. Phytopathol. Soc. Japan* **22**: 75-78.
- Tomiyama, K., and N. Takase. 1956. Physiological studies on the defense reaction of

- potato plant to the infection of *Phytophthora infestans*. I. Changes in the physiology of potato tuber induced by the infection of *Phytophthora infestans* and their varietal differences. *Hokkaido Natl. Agr. Expt. Sta., Research Bull.* **71**: 32-50.
- Tomiyama, K., N. Takase, R. Sakai, and M. Takakuwa. 1955. Physiological studies on the defense reaction of potato plant to the infection by *Phytophthora infestans*. II. Changes in the physiology of potato tuber induced by the infection of the different strains of *Phytophthora infestans*. *Ann. Phytopathol. Soc. Japan* **20**: 59-63.
- Tomiyama, K., M. Takakuwa, N. Takase, and R. Sakai. 1956. Physiological studies on the defense reaction of potato plant to the infection of *Phytophthora infestans*. III. The influence of pre-infectional ethanol narcosis upon the physiological reaction of potato tuber to the infection of *Phytophthora infestans*. *Ann. Phytopath. Soc. Japan* **21**: 17-22.
- Tomiyama, K., R. Sakai, N. Takase, and M. Takakuwa. 1957. Physiological studies on the defense reaction of potato plant to the infection by *Phytophthora infestans*. IV. The influence of pre-infectional ethanol narcosis upon the physiological reaction of potato tuber to the infection by *Phytophthora infestans*. *Ann. Phytopath. Soc. Japan* **21**: 153-158.
- Toyoda, S., and N. Suzuki. 1957. Histochemical studies on the lesions of rice blast caused by *Piricularia oryzae* cav. III. Changes in the respiration of infected tissues. *Ann. Phytopath. Soc. Japan* **22**: 173-177.
- Turner, J. S. 1951. Respiration (The Pasteur-effect in plants). *Ann. Rev. Plant Physiol.* **2**: 145-168.
- Uritani, I., and T. Akazawa. 1953. Phytopathological chemistry of black-rotten sweet potato. Part 12. Activation of the respiratory enzyme systems of the rotten sweet potato. *J. Agr. Chem. Soc. Japan* **27**: 789-795.
- Uritani, I., and T. Akazawa. 1955a. Antibiotic effect on *Ceratostomella fimbriata* of ipomeamaron, an abnormal metabolite in black rot of sweet potato. *Science* **121**: 216-217.
- Uritani, I., and T. Akazawa. 1955b. Biochemical studies on sweet potato infected with black rot. *Kagaku (Tokyo)* **25**: 614-620. (In Japanese.)
- Uritani, I., and I. Hoshiya. 1953. Phytopathological chemistry of the black-rotten sweet potato. Part 6. Isolation of coumarin substances from sweet potato and their physiology. *J. Agr. Chem. Soc. Japan* **27**: 161-164.
- Uritani, I., and K. Iechika. 1953. Phytopathological chemistry of black-rotten sweet potato. Part 9. Some knowledges concerning ascorbic acid in the rotten sweet potato. *J. Agr. Chem. Soc. Japan* **27**: 688-692.
- Uritani, I., T. Akazawa, and M. Uritani. 1954. Increase of respiratory rate in sweet potato tissue infected with black rot. *Nature* **174**: 1060.
- Uritani, I., T. Akazawa, and M. Uritani. 1955. Phytopathological chemistry of black-rotten sweet potato. Part 18. Respiration and colour change of the rotten sweet potato. *J. Agr. Chem. Soc. Japan* **29**: 344-349.
- Vager, R. N. 1955. Changes in activity of respiratory enzymes in virus infected plants. (Orig. in Russian.) *Zhur. Obshchei Biol.* **16**: 298-305; *Chem. Abstr.* **50**: 2763.
- Walker, J. C., and M. A. Stahmann. 1955. Chemical nature of disease resistance. *Ann. Rev. Plant Physiol.* **6**: 351-366.
- Weimer, J. L., and L. L. Harter. 1921. Respiration and carbohydrate changes in sweet potatoes by *Rhizopus tritici*. *J. Agr. Research* **26**: 627-635.

- Wheeler, H., R. R. Romanko, and L. R. Krupka. 1958. Personal communication.
- Yabuta, T., K. Kambe, and T. Hayashi. 1934. Biochemistry of Bakanae-fungus. I. Fusarinic acid, a new product of the Bakanae-fungus. *J. Agr. Chem. Soc. Japan* **10**: 1059-1068.
- Yamaguchi, A. 1958. Respiratory increase of *Nicotiana glutinosa* infected by tobacco mosaic virus. *Ann. Phytopathol. Soc. Japan.* **25**: 28.
- Yamaguchi, A., and T. Hirai. 1956. Nature of virus infection in plants. (IV) Dehydrogenase activity of green leaves during the course of virus infection. *Virus (Osaka)* **6**: 1-7.
- Yarwood, C. E., and M. Cohen. 1951. Hypertrophy from the uredial stage of bean rust. *Botan. Gaz.* **112**: 294-300.
- Yarwood, C. E., and L. Jacobson. 1950. Selective absorption of Sulphur-35 by fungus-infected leaves. *Nature* **165**: 973.
- Yarwood, C. E., and L. Jacobson. 1955. Accumulation of chemicals in diseased areas of leaves. *Phytopathology* **45**: 43-48.

## CHAPTER 11

# Histology of Defense in Plants

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Disease resistance in plants is understood to be a condition in which the plant, when attacked by a pathogen, suffers little or no injury. This subject has been treated recently under two headings: resistance to penetration, and resistance to disease development and spread (Kawamura and Ono, 1948). The former applies to all structures which oppose the penetration of the pathogen, and the latter to the condition as controlled chiefly by protoplasmic activity in the cell itself (functional resistance) (Tochinai, 1951). In the former case, the mechanical characteristics of superficial layers play a principal role. Resistance in plasma of epidermal cells of a physiological nature is also involved. In the latter case, we are chiefly concerned with the plasmatic defense reaction. Although not completely synonymous, this term is often used in place of immune reaction (Gäumann, 1950).

In the present chapter, we shall discuss the defensive phenomena in disease incidence from histological and cytological viewpoints. Defense mechanisms will be interpreted here principally as having two meanings: (1) static anti-infectious structures of superlying layers existing prior to the infection, and acting mainly as a barrier to penetration, and (2)



dynamic defense structures that appear in tissues postinfectiously as a response to pathogenic invasion and which impede further spread of the pathogen.

## I. STATIC ANTI-INFECTIONOUS STRUCTURES

The mode of invasion of the host by the pathogen generally occurs by one of two methods: (1) penetration directly through the cuticle of outer epidermal cell walls, and (2) intrusion through the natural openings such as stomata, lenticels, and wounds. Therefore, the quality of epidermal walls, structure of stomata and lenticels, and the characters of cytoplasm in epidermal cells may be enumerated as hindering to infection. The latter, however, will be considered later.

### A. Superficial Structure as a Factor in Resistance to Penetration

A plant body is passively protected against pathogens by its superficial covering layers. These covering layers usually consist of cuticle and epidermis. Epidermis is sometimes covered with a wax layer, and the cell walls of epidermal layers often undergo a suberization or lignification that acts as a barrier to penetration. In cereals, the silification of epidermal cell walls occurs frequently.

#### 1. Structure of Epidermal Cells

Young blades of cereal plants and fruits of the genus *Prunus* are usually covered either with a waxy layer or with hairs. By making it more difficult for infection drops to adhere to them, the wax or hairs aid indirectly in resistance to penetration. However, not uncommonly, the removal of wax from leaves has no effect on infection, and hairs do not always make infection difficult.

Cuticle and the outer wall of epidermal cells may directly impede the entrance of pathogens. In this case, the thickness and toughness of cell walls are of importance, although some investigators believe that the fungus does not make its way through cell walls or even cuticle by mechanical means alone. The germinated basidiospores of most rust fungi penetrate through the cuticle into the interior of leaf (cuticular invasion). However, the tough epidermal outer wall and cuticle of leaves make direct penetration by a given pathogen more difficult and serve as barriers against invasion. It is clear from Table I that species of *Berberis* not susceptible to black rust (*Puccinia graminis*) resist sporidial invasion, because of the thick cell walls, even when leaves are young (Melander and Craigie, 1927). Thus, plants with tough epidermal walls are not attacked by a given pathogen, and display resistance to penetration.

However, these same plants will display a high degree of susceptibility when wounded if their inner tissues are sensitive to the pathogen.

Some varieties of Japanese pear (*Pyrus pyrifolia* var. *culta*) express a high degree of susceptibility to the infection of *Alternaria kikuchiana*. Germ tubes of conidia usually penetrate directly through the cuticle and enter the inner tissues of leaves. Under field conditions, incidence of this disease on mature shoots is low, although they are highly susceptible (Torigata, 1957). The epidermal cell wall of mature leaves is always thick and tough. Possibly, epidermal structures play a minor role in resistance to fungus attack, and resistance to this disease in resistant varieties resides in the cell as a result of plasmatic defense.

TABLE I  
AVERAGE THICKNESS OF EPIDERMAL CELL WALLS OF LEAVES  
OF CERTAIN *Berberis* SPECIES<sup>a</sup>

Species	Thickness of outer epidermal wall and cuticle (in microns)	
	Leaves 2-3 days old	Mature leaves
Highly susceptible		
<i>Berberis canadensis</i>	0.88	1.29
<i>Berberis dictyophylla</i>	0.82	1.80
<i>Berberis vulgaris</i>	1.10	1.87
Slightly susceptible		
<i>Berberis brachypoda</i>	1.43	2.56
<i>Berberis lycium</i>	1.23	3.41
<i>Berberis pruinosa</i>	1.16	2.20
Not susceptible		
<i>Berberis thunbergii</i>	1.57	2.44
<i>Odostemon repens</i>	1.75	3.01

<sup>a</sup> After Melander and Craigie, 1927.

In susceptible varieties of flax, in general, the epidermis lacks a well-developed cuticle, the individual epidermal cells are rectangular rather than isodiametric, and the hypodermis is usually absent. Consequently, resistance to puncture of the epidermal membrane may be correlated with a well-developed cuticle, shape of the individual epidermal cells, and the presence of hypodermis. Thus, the varieties of flax resistant to rust (*Melampsora lini*) possess a tougher epidermis than susceptible varieties do (Sharvelle, 1936). The strength of the epidermal membrane in certain varieties may determine the ability of the rust fungus to break

out and liberate its uredospores. Such a condition may reduce the amount of available inoculum produced in the course of a summer.

Blast disease causes serious damage to the rice crop every year. Its causal fungus, *Piricularia oryzae*, invades directly through the epidermal wall (Fig. 1). The motor cells and the guard cells of stomata are the pathway through which the fungus penetrates most easily. As indicated

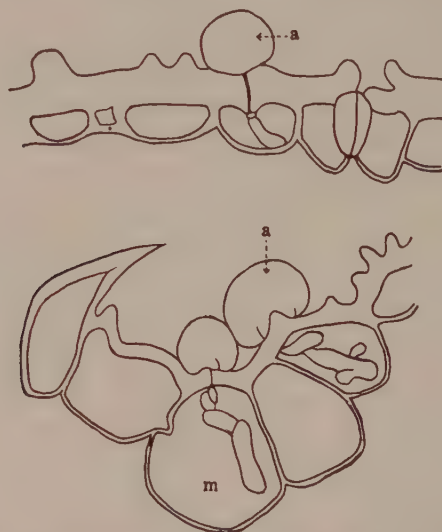


FIG. 1. Mode of entrance into the epidermal cells of rice leaves by *Piricularia oryzae*: (a) appressorium, (m) motor cell. (After Yoshii, 1936.)

TABLE II  
PENETRATING HYPHA FORMATION FROM APPRESSORIA FORMED  
ON EPIDERMAL CELLS OF KAMEJI RICE NO. 3<sup>a</sup>

Epidermal cell	Per cent appressoria formed	Per cent penetrating hypha formed
Motor cells	53.0	63.7
Long cells	16.2	7.9
Short cells	12.8	6.1
Hairs	1.7	—
Stomata	6.0	—
Short cells contacted with stoma	7.7	15.1
Midlamella between stoma and short cell	2.6	6.1

<sup>a</sup> After Ito and Shimada, 1937.

in Table II, the entrance of the fungus in more than half of the total invasions is through motor cells. It has been considered that the lignification of the outer wall of motor cells does not take place rapidly, being kept in a pectocellulosic condition for a long time, while most of the other epidermal cell walls are cellulosic and become lignified sooner (Fig. 2, 1) Yoshii, 1936). Hashioka (1950) confirmed the lignification in epidermal cell wall of rice leaves of differing age by the application of Mäule's reaction. According to his results, positive reaction to lignification in epidermal cell walls was obtained after 60 days of development of the leaf blades. With the exception of motor cells, the histochemical tests of Suzuki *et al.* (1953) show that chlorogenic acid appears to accumulate in walls of epidermal cells usually combined with a certain con-

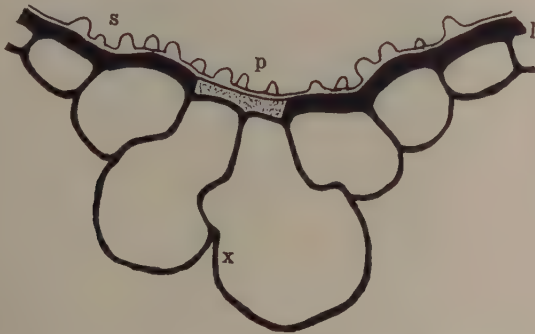


FIG 2. The cross section of motor cells in leaves of rice plants. (1) lignified cell wall, (p) pectin layer (dotted area), (s) silicic acid deposited layer, (x) cell wall of motor cell, on which silicic acid deposits easily. (After Yoshii, 1936.)

stituent of cell membrane. In walls of motor cells, either there is no lignification, or lignin does not deposit sooner, thus at least partially accounting for the more ready penetration by the fungus through motor cells.

It is a well-known fact that silicic acid content of leaves is inversely related to incidence of diseases of rice. Usually, silicic acid is deposited abundantly on lignocellulosic walls of epidermal cells (Yoshii, 1936) and the deposition increases with the maturity of leaves. Under favorable conditions at maturity, silicic acid is heavily deposited in motor cells, and this increases resistance to fungus penetration (Fig. 3).

Planting rice plants in flooded soil is the usual cultural practice in Japan. This procedure promotes the deposition of silicic acid on cell walls and prevents fungal invasion.

Low air and soil temperature reduce both the thickness of the outer



wall of epidermal cells and the deposit of silicic acid on epidermal cell walls. The thinner walls and the decreased deposit of silicic acid are perhaps the factors which cause severe incidence of blast disease and *Helminthosporium* blight (Hashioka, 1950; Suzuki, 1951).

In a discussion of mechanical barriers to fungal penetration, the strength of the epidermal membrane to puncture is usually considered. For this purpose, the Jolly balance is adapted (Hawkins and Harvey, 1919; Melander and Craigie, 1927). As has been cited above, the pressure required to puncture the epidermis of leaves and stems of flax varieties varies with their reaction to flax rust. Highly resistant varieties

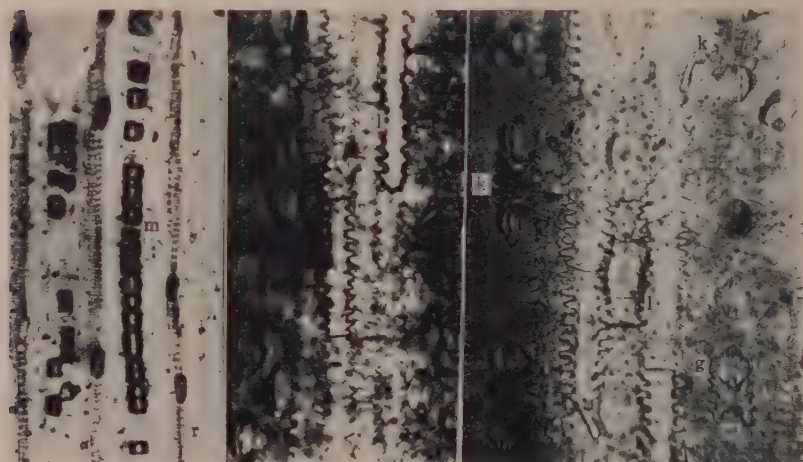


FIG. 3. Ash-figures of leaves of rice plants. Silicated epidermal cells: (g) guard cell, (k) short cell, (l) long cell, (m) motor cell, (r) rice cell.

require more pressure than the susceptible ones (Sharville, 1936). Ito and Sakamoto (1939), measuring the resistance of the epidermis of rice leaves to needle puncture, found that feeding silicic acid to rice plants increased the resistance to puncture. In turn, this resistance to puncture was correlated with a reduction in the incidence of blast disease. Yoshii (1941a), however, aimed to measure the toughness of the cell wall of motor cells, and obtained a result opposite to that of Ito and Sakamoto. His results showed that the resistance to needle puncture decreased with the increase in the supply of silicic acid. In this case, the total amount of silicic acid in the plant increased and the incidence of blast disease decreased (Table III).

Yoshii (1941b) also tested the toughness of leaf, when the plants

TABLE III  
STRENGTH OF EPIDERMAL MEMBRANE TO NEEDLE PUNCTURE IN  
LEAVES OF RICE PLANTS AND TOTAL AMOUNT OF SILICIC ACID<sup>a</sup>

Silicic acid given (mg. per liter)	Strength of epidermis (gr./mm. <sup>2</sup> )			Total amount of silicic acid (%)
	2nd leaf <sup>b</sup>	3rd leaf <sup>b</sup>	4th leaf <sup>b</sup>	
0	902.3	813.3	777.9	1.8
50	863.3	792.6	766.1	3.5
250	847.8	808.1	755.8	10.2
500	840.1	793.1	753.4	12.9

<sup>a</sup> After Yoshii, 1941a.

<sup>b</sup> From upper level.

TABLE IV  
THE TOUGHNESS AND SILICIC ACID CONTENT OF LEAVES OF RICE  
GROWN UNDER FIELD CONDITIONS AND SUPPLIED WITH  
DIFFERENT AMOUNT OF NITROGEN AND SILICIC ACID<sup>a</sup>

Nitrogen <sup>b</sup> supplied (kg. per 0.1 hectare)	Silicic acid supplied (kg. per 0.1 hectare)	Total content of silicic acid (SiO <sub>2</sub> ) (% dry matter basis)	Toughness of epidermis (gm./mm. <sup>2</sup> )	Incidence of blast disease
18.75	262.5	6.71	719.9	±
	0.0	3.74	938.3	++
11.25	262.5	8.90	916.1	±
	0.0	5.97	960.5	±
3.75	262.5	10.93	941.7	—
	0.0	8.46	1055.2	—

<sup>a</sup> After Yoshii, 1941b.

<sup>b</sup> Ammonium sulfate.

were supplied with silicic acid and ammonium sulfate. Evidently nitrogen affects toughness of the leaf (Table IV). However, there is no relationship between silicic acid content and toughness of the leaf.

## 2. Structure of Stomata and Lenticels

Except for bacterial diseases there is probably no evidence that infection is prevented by the structure of the stomata.

In citrus canker, caused by a bacterium (*Pseudomonas citri*), the bacteria are not able to attack the dry cutinized or waxy cell walls of citrus. In intact leaves, the bacteria can enter only through the stomatal openings. Szinkum mandarin (*Citrus nobilis* var. *Szinkum*) possesses a

resistance to citrus canker, whereas certain kinds of grapefruit (*Citrus grandis*) are very susceptible. Hence, resistance to penetration must be involved in the structure of stomata (McLean, 1921). Each has stomata of similar size, but they differ mainly in the entrance ridge. In the resistant variety, Szinkum mandarin, the stomata have an extremely narrow entrance with broad lips over the stoma, whereas in the Florida grapefruit the stomata have broad oval lips. In the mandarin this structure may practically exclude water from the stomata, whereas water can more readily enter the stomata of the grapefruit. This exclusion of water is sufficient to account for the resistance of certain citrus varieties to canker.

Possibly in the rusts, the uredospore germ tubes enter plants through stomata. However, as shown by Hart (1929), the stomata of some wheat varieties are closed much of the time and the stem rust, *Puccinia graminis tritici*, cannot usually force its way through closed stomata. She termed this as "functional resistance." The delayed opening in the morning of stomata of resistant wheats, prevents the entry of the stem rust until the moisture on the plant has evaporated, thus exposing the delicate germ tubes to desiccation and death. Closed stomata offer no effective barrier to the entry of leaf rust, *Puccinia triticina*, which never enters through open stomata because of the prompt stomatal closure in response to appressorium formation (Caldwell and Stone, 1936).

Certain other fungi are not able to push their way through closed stomata. *Cercospora beticola* can enter sugar beet leaves only through open stomata. The maturity of the leaf is important and infection is closely related to stomatal movement. Young leaves are not infected because stomatal movement is not active. Mature leaves are attacked severely, but old leaves are not invaded because movement is feeble (Pool and McKay, 1916).

Lenticels, before they become suberized, are sometimes good portals for invasion by pathogenic fungi. *Actinomyces scabies* (*Streptomyces scabies*), the cause of common potato scab, enters through lenticels of young tubers and stems (Lutman, 1945), although it can also invade through stomata and wounds. The cells of young lenticels are usually round and loosely arranged with rather large intercellular spaces. When the filaments of the scab organism grow among these cells, the lenticel meristem is stimulated to divide actively and to form closely packed, radially elongated cells. This appears to be an attempt at cork formation. These cells do not, however, usually constitute a barrier to the further extension of the pathogen because they are not suberized. They do not form a true cork layer, and the scab organisms continue their invasion.

Apparently, the pathogen tends to delay suberization of the cells in the lenticels (Jones, 1931; Longrée, 1931; Darling, 1937).

The lenticels of healthy mulberry trees conceal mycelia of various fungi, some 30–60% of which are pathogenic to mulberry trees (Aoki, 1941). Among these are *Diaporthe nomurai*, the cause of the devastating mulberry blight found in snowy regions, and *Gibberella lateritium*, the cause of bud blight.

The normal lenticels have a cork cambium at the base which is connected with the periderm of the shoot. The cork cambium divides forming closing layers and complementary cells alternately, and the fungi may be found in the vacuity among these cells.

Under normal conditions when the plants are growing vigorously, the fungi in the lenticels are not able to invade through the cork layers into the interior. However, under unfavorable conditions, the pathogenic fungi accomplish the infection by passing through the cork layers of weakened lenticels. This is why mulberry blight occurs in the heavy snow region, where the plants are buried for a long time under heavy snow cover and lose their vigor.

The fungal components of the lenticels vary with the variety of mulberry, depending on the structure of the lenticels. In general, the rough structured lenticels have more fungi than the closed type. The correlation between resistance to mulberry blight and number of fungi isolated is inverse. A large number of fungi were isolated from the lenticels of resistant varieties, but a few can penetrate the cork cambium. On the other hand, only a small number of fungi were isolated from the lenticels of susceptible varieties, but a large percentage of them were able to penetrate the cork cambium of the lenticels (Aoki, 1945).

#### B. Mechanical Barricade Tissues as a Factor in Resistance to Invasion

Mechanisms that interfere with invasion of the host are (1) the static resistance to spread, already present prior to infection, and (2) the dynamic defense reactions which become apparent after infection occurs (Gäumann, 1950). In the present section we shall deal with the former from the histological viewpoint.

Resistance to invasion is sometimes associated with histological characteristics of walls. This resistance opposes the progress of pathogens and may be distributed throughout the tissues or localized in certain barricade tissues.

The epidermis of wheat varieties consists of a single layer of cells, the inner walls of which are sometimes lignified. Just beneath the epi-



dermis there is chlorenchymatous collenchyma. This tissue sometimes extends in an almost continuous band around the entire stem, although it usually is interrupted by the strands of sclerenchyma. The collenchyma cells, then, are aggregated into isolated bundles, the size and number of which vary considerably in different varieties. In Kota, the sclerenchyma fibers divide the collenchyma into distinct areas, while in Little Club the sclerenchyma is much less conspicuous and the collenchyma is practically continuous. Marquis stems have somewhat more sclerenchyma fibers than the Little Club stem; in the stem of Sonem emmer a large amount of sclerenchyma is always developed. The collenchyma areas in this variety are extremely small and the sclerenchyma area is decidedly predominant, making up the major portion of the stem proper. Less infection with both *Puccinia triticina* and *P. graminis* takes place on stems of *Sonem emmer* than of any other wheat (Hursh, 1924). However, under epidemic conditions, a large number of individual infections may result even on varieties with extensive sclerenchyma. Under such conditions, the susceptible Little Club and Marquis varieties are seriously injured. The structure of the stem affects only the extent of the spread of rust fungi and its subsequent rupture of the epidermis. However, resistance to stem rust must be considered as being due fundamentally to a plasmatic defense.

Rice leaf smut, caused by the infection of *Entyloma oryzae*, shows a black, short, linear symptom limiting itself usually between two veins of leaves. Thus, the mechanical tissues affect the extent of the spotted area. Fertilizer affects greatly the development of the mechanical tissues, especially when upland rice plants are cultivated under flooded conditions (Shimada, 1957).

## II. DYNAMIC DEFENSE REACTION

In contrast with the defensive structure, the dynamic defense reaction is evoked postinfectionally by response to the stimulus of infection, on the one hand forming histological barricades, and on the other revealing protoplasmic defense in the cell itself. In some cases, these host responses may be considered an inflammatory reaction. However, this discussion will be limited to situations where infection by pathogenic organisms is prevented.

The defense reaction can be classified according to its origin: (1) the autonomous antiparasitic defense reaction, and (2) the induced defense reaction. We shall deal here with the former only. According to Gäumann (1950), the antiparasitic defense reaction (anti-infectious defense reaction) is the reaction of cells aimed directly at the pathogen and intended to weaken and destroy it. The existence of this reaction in cells is shown

by the fact that most infectious plant diseases, not systemic, do not spread indiscriminately through the host. The curves in Fig. 4 actually show this. Thus, after a certain time, the disease intrusion comes to a standstill. Infection remains localized, giving to a disease its characteristic symptoms.

We have shown above, that there are some cases where outer epidermal layers and mechanical barricade tissues are capable of preventing pathogenic invasion. However, if a pathogenic organism should make its way into the interior of plant tissues by passing through these barricades,

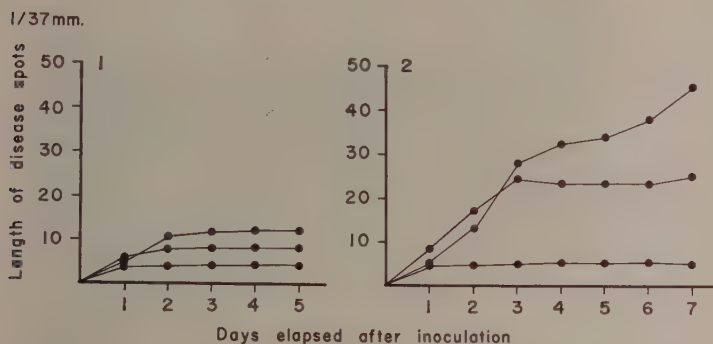


FIG. 4. Curves of developing areas in spots revealed in leaves of rice plants due to the infection of *Cochliobolus miyabeanus*. (1) supplied with a large amount of ammonium sulfate, (2) supplied with normal amount of fertilizers. (After Kurosaki, 1957.)

the defense reaction against further intrusion by the pathogen may be induced autonomously in the focus of the invasion point. Then, the autonomous defense may appear: on the one hand as a histological barrier which acts to demarcate the infected lesion (histogenic defense reaction), or, on the other, as a plasmatic activity in the cell itself.

First, we shall deal with the histogenic reaction.

#### A. Histogenic Defense Reaction

Defense reactions which are exhibited histologically will be considered in this section. Defense of this type may be manifested as a demarcation of the infected lesions and as a callus-like swelling of the membrane. Both serve to prevent further intrusion of the pathogen. Gäumann (1950) has stressed the antitoxic effect in some cases of demarcation. This involves: (1) demarcation of infected lesions by forming cicatricial layers, abscission cells, tyloses, or gum; (2) callus-like swellings or callosities formed on the wall.

### 1. Demarcation of Infected Lesions

After infection, the invaded tissue is sometimes demarcated histologically. In some cases, this results from cork layer formation and in others from gum deposition or from formation of abscission cells.

a. *Cicatricial Layers*. In some plants, suberized healing tissues develop, demarcating the localized lesions of infection. This may lead to scabbing. Metabolic products secreted by the causal organism may stimulate the formation of this cicatricial demarcation. Fungi of the

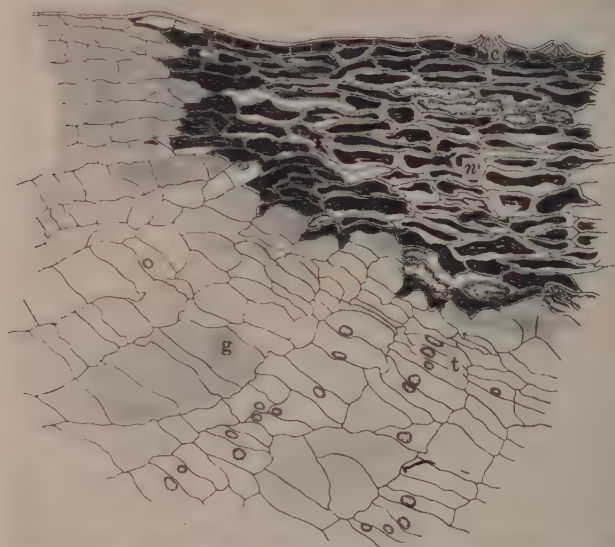


FIG. 5. Renewed meristem formed around the necrotic lesion of fruit of grape invaded by *Elsinoe ampelina*: (c) conidial sorus, (g) cells showing granular deposits, (n) necrotic cell group, (t) renewed meristem.

genus *Sphaceloma* are especially able to induce cork cells to form in various plants and give them a characteristic scabby appearance on the surface. As Gäumann (1950) suggested, the cicatrice may cut off the influence of toxic substances of the fungi, which may diffuse from the infected area. He designated this phenomenon as the antitoxic defense reaction. An anthracnose disease of grape infected by *Elsinoe ampelina* and citrus scab caused by *Elsinoe fawcettii* are representatives of this type. *Sphaceloma ampelinum*, the conidial stage of *Elsinoe ampelina*, attacks every part of the plant. On fruits, the first noticeable symptom is

a minute round speck on the surface. With gradual increase in size of spots, the peripheral region more or less bulges out accompanied by the formation of a depression of the central area. Renewed meristematic activity occurs in cells of the peripheral zone of the spots. From this meristem (Fig. 5, t) cork cells may be formed (Akai, 1951). On soybean pods, a suberized barricade delineates the area infected by *Sphaceloma kurozawana*. At first, the cytoplasm degenerates and turns brown in the invaded epidermal cells, whereupon the hyphae do not enter into the palisade tissue. Thereafter, the proliferation of palisade cells takes place under the infection focus, thus making a scabby appearance.

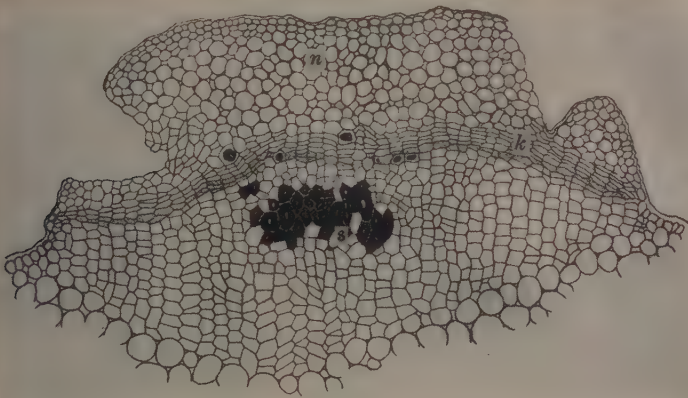


FIG. 6. The postinfection cork layers in the lesions of citrus melanose on the stem of grapefruit: (k) renewed phellogen, (n) necrotic cells, (s) stone cells (not a response to this invasion). (From Akai, 1950.)

The melanose disease, caused by *Phomopsis citri* (*Diaporthe citri*) develops on leaves, stem, and fruits of citrus, where it causes abundant small black spots. Although there is no corky appearance to the unaided eye, a perfect demarcation of the infected zone is produced by the post-infection cork layers (Fig. 6). On artificially wounded fruits, the pathogen is forced to express a stem end rot symptom that causes the entire fruit to break down. However, when uninjured fruits are used, typical melanose spots result. The band of cork layers may effectively cut off the growth of the mycelium and prevent further invasion of the causal fungus toward the interior of the fruit. The region bulges out, but not noticeably so, due to hyperplasia of cells. The cells outside the cork layers collapse and turn brown, showing a granular fatty degeneration of the cytoplasm (Bach and Wolf, 1928).



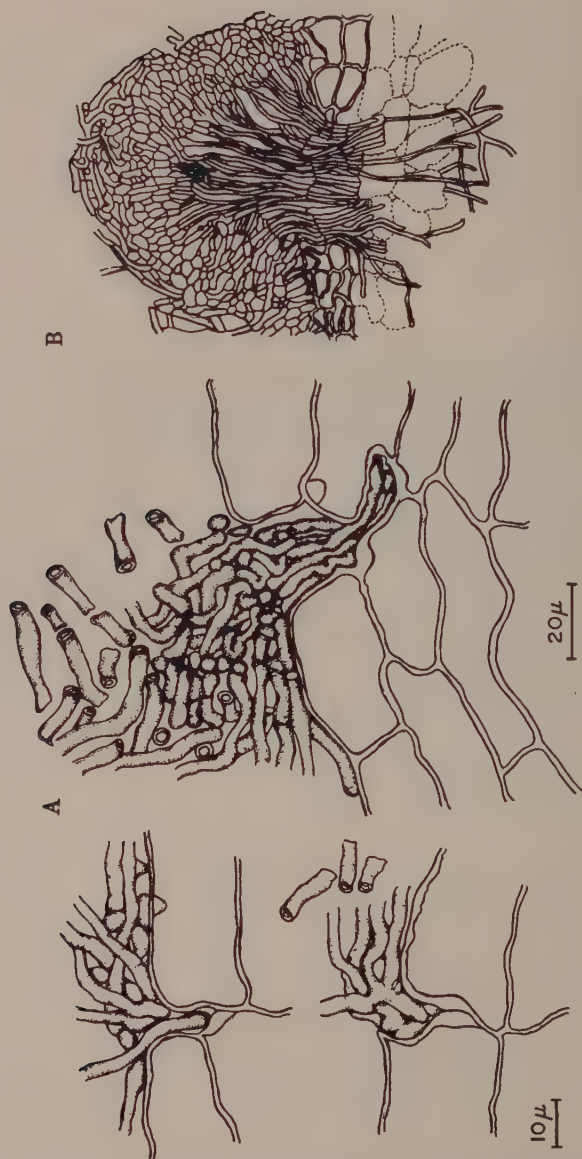


FIG. 7. The infection cushion of *Helicobasidium mompa* derived from the mycelial strand, passing through the cork layers and dissolving the starchy parenchyma of sweet potato tuber. (After A: Ito, 1949; B: Suzuki *et al.*, 1957.)

The destructive "Murasaki-Mompa" disease caused by *Helicobasidium mompa* causes rot of underground parts of many plants, some 45 families, 76 genera, and 104 species (Ito, 1949). The basal part of stems and fleshy roots of apple, mulberry trees, and sweet potatoes are among those that are severely attacked. On sweet potatoes, the mycelium of the fungus grows epiphytically for a long time as a purplish, felt-like network of rhizomorphs. During this period, hyphae penetrate into the middle lamellae of the cork layer cells, but not so deeply as to pass through the cork layer (Suzuki *et al.*, 1957).

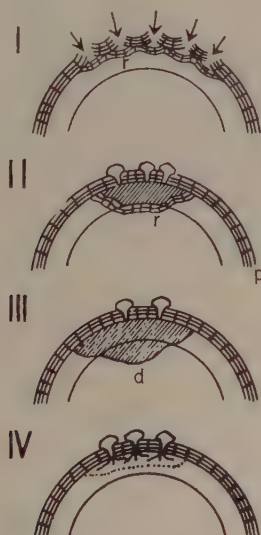


FIG. 8. Types of reaction in tubers of sweet potato against *Helicobasidium mompa*: (d) degenerated zone, (p) periderm, (r) postinfectionally renewed cork layers. (After Suzuki, 1957.)

The penetrating hyphae (Fig. 7) gradually develop into a bundle and finally into an infection cushion (Ito, 1949; Suzuki *et al.*, 1957). Thus, the hyphae of the cushion gain entrance into the starchy parenchyma and cause rot of the tubers.

Defense of the tubers against fungal invasion is observed most actively during the period of rapid growth. In response to invasion, the cork cambium develops layers of suberized cells that are more than six layers deeper than normal. Infection in this disease is of the following four types (Suzuki, 1957):

Type 1. The invasion hyphae are inhibited from reaching the starchy

parenchyma by rapid and successive formation of cork layers. This causes sloughing off of the infected zones (Fig. 8-I). Host cells degenerate, turn brown and enclose the tips of the hyphae. There is no change in appearance of the tuber to the unaided eyes.

Type 2. The starchy parenchyma beneath the infection cushion displays a brown rot appearance, and the post infection cork layers (Fig. 8-II, r) inhibit further invasion of the hyphae by enclosing the brown rotted area. Thus, a complete demarcation of the lesion takes place.

Type 3. A rapid change in light brown color of starchy parenchyma occurs under the infection cushion. This change develops widely, finally causing a soft rot of tubers.

Type 4. Starchy parenchyma is macerated without change in color by intensive pectolytic activity of the fungus.

Type 1 represents the most active defense against fungal attack. If the fungus passes through the barrier, the second type of defense may be induced. The third and fourth types may occur in the susceptible condition of tubers.

Lignification of cells takes place in the periphery directly in contact with the rotted brown zone, and two to three layers away from this peripheral lignification, a second lignification occurs in cells. When the lignification does not occur, the rotting of tissues proceeds rapidly. However, the cork may be most stable against the action of this fungus. Therefore, when the disease proceeds gradually, it is checked almost completely by the newly formed cork cell layers. The lignified cells, however, do not check the disease completely as do the cork cells. Usually, the second type of defensive cork layers develops in the periphery of the necrotic zone, surrounding the lignified cell layers, when tissues are resistant. Cork layers do not form in susceptible tissues.

At the first stage of infection, the middle lamellae of cork layer cells are penetrated by the fungus, as has been shown before. Pectic material in the middle lamellae swells when the hyphae come in contact with it and the pH value is decreased. After passing through the cork layers, the fungus comes into contact with the starchy parenchyma. In susceptible varieties, these tissues are then macerated by the action of fungal enzymes. Itaconic acid is isolated from such tissues (Araki *et al.* 1957). The decrease of pH in these tissues is mainly due to the accumulation of chlorogenic acid and caffeic acid, and to itaconic acid produced by the fungus. When treated with ruthenium red in the early stage, the pectic substance of the invaded tissues—in contact with the hyphae—is stained yellow, and the tissues beneath the phellogen are stained carmine red.  $\text{FeCl}_3$ -potassium ferricyanide solution stains pectic materials in the

cork layers blue. No color reaction occurs in pectic materials produced postinfectionally.

Lignification of the cell membrane is accelerated by infection. The red color reaction of cell membranes treated with phloroglucin-HCl is chiefly due to lignin, although caffeic and galacturonic acids react similarly (Suzuki, 1957).

Corky demarcation appears on various other plant diseases. In chestnut blight, caused by *Endothia parasitica*, trees often develop a cork barrier (Bramble, 1936; Bazzigher, 1957). Infection by *Actinomyces scabies* causes a scabby appearance on the surface of developing potato tubers. Similarly, *Cladosporium carpophilum* causes a corky appearance on the surface of peach fruits. In apples and pears, once the fire blight lesions are corked off, the cork layers and the xylem commonly serve as relatively effective barriers against further invasion of the causal bacteria (Shaw, 1934). In the diseased leaves and petioles of *Aralia cordata* and *Fatsia japonica* infected by *Elsinoe araliae*, cork layers very similar to those found on the diseased stem of grape are formed surrounding necrotic spots. If the fungus invades further, after the cork layers are formed, underlying collenchyma cells undergo a rapid lignification. The lignification of bast fibers beneath the diseased spot takes place more rapidly than in uninfected tissues.

A very definite cicatrice forms at the margin of the necrotic area of leaves of *Prunus domestica* attacked by *Coccomyces prunophorae*. The healing tissue of wound periderm consists of several layers of cells. The phellem is made up of a mass of large cells, whose thick walls are not only suberized, but also lignified. The cells lying nearest the necrotic lesion become filled with a dense granular substance resembling tannin, while the remaining cells are apparently devoid of contents. The epidermal cells of this layer also have suberized walls. Both phellogen and phelloderm are present in the wound periderm. The cells comprising these two layers are thick walled, but this thickening is entirely cellulosic in nature. Chloroplasts are absent from these cells. The phellogen consists of a single layer of cells which are filled with dense protoplasm, while the cells of the phelloderm contain only a peripheral layer. The periderm ties between the two epidermis of the leaf (Fig. 9) and isolates perfectly the diseased portion (Cunningham, 1928).

Cork layers around the necrotic lesions have been noted in leaves of sugar beet infected by *Cercospora beticola*; in *Paulownia* twigs infected by *Gloeosporium kawakamii*; in apple leaves attacked by *Physalospora cydoniae*; in cankered twigs of apple caused by *Valsa mali*; in portions of twigs of cherry or peach showing dieback caused by *Valsa japonica* or



*Leucostoma personii*; in geranium stem rot caused by *Pythium com-  
plectens*. In tobacco plants the rapidity of cork formation beneath the  
lesions due to the attack of *Thielavia basicola* is an accurate criterion of  
its resistance (Conant, 1927). In leaves of *Nicotiana glutinosa* infected  
with tobacco mosaic virus, a corky barricade is also formed around the  
necrotic spots (Yoshii and Kawamura, 1947).

Sweet potatoes have the ability to form cork layers covering wounds  
when the environmental conditions are favorable (Weimer and Harter,  
1921). The wound cork layers may be healing tissue, but they sometimes  
serve as a defensive mechanism against the invasion of pathogens. In



FIG. 9. Cork layer at the edge of a lesion on a leaf of *Prunus domestica* caused by *Coccomyces prunophorae*. (After Cunningham, 1928.)

cut tubers of potatoes, the outer walls of living cells suberize at the cut  
surface and afterwards wound periderm may be formed. This prelim-  
inary suberization in cell walls has been termed the pseudocicatrice  
(Wylie, 1930, 1931). Generally, the first effect is defensive lignification  
of cells surrounding the wound; and deposition of wound gum takes  
place. At the inner side of this barricade, a set of cells lose their reserve  
nutrient and chloroplasts and form a renewed meristem that divides to  
form cork cells. From the resulting differentiation of cork mother cells,  
the five or ten layers of cork cells are formed by division on the outer  
side. These cork barriers are formed most vigorously under conditions  
ranging between 30°–35° C. and 90–95% relative humidity. Under exces-  
sive humidity (90–100%) cork formation is retarded, while below 80%  
no cork is formed in sweet potato (Yoshii, 1944). In cut tubers of pota-  
toes the optimum temperature for suberization of superficial cells and

formation of wound periderm is between 21° and 35° C. (Artschwager, 1927).

Soil moisture may be one of the factors influencing cork formation in tubers. Without doubt, water-logged soils favor the growth of the soil-borne pathogens, especially the facultative anaerobes. An excess soil moisture obviously inhibits cork formation and enhances the incidence of the disease, e.g., blackleg of potato. Therefore, the presence of air (oxygen) may be necessary for the formation of cork cells (Jahrmann, 1913; Leach, 1931).

Wound cork layers are found in the mulberry root. This cork is formed during the growing season, as in stems, starting from meristematic cells near the cambium, but not from the lignified bast fibers or woody tissues. These layers, to some extent, prevent the spreading of the disease caused by *Roselinia necatrix*, but when the mycelial strand reaches the cork layers, it usually ruptures them (Sakurai, 1952). Thus, cork layers are sometimes of no value as a barrier to the invasion of the pathogen. The entrance of *Cylindrocarpon ehrenbergi* occurs by direct penetration of the cork covering in roots of alfalfa and sweet clover. The hyphae mass up and push their way between the cork cells in an apparently mechanical manner (Cormack, 1937).

b. *Abscission Layers*. Whatever the cause may be, the spots produced on leaves of stone fruit plants slough out. Many fungi and bacteria, pathogenic to these plants, produce such a shot-hole effect. Moreover, the perforation may be formed by wounds or sprays containing a dilute (0.01 M) copper sulfate solution. Leaves of peach trees planted under copper wires are very easily perforated by the copper leached from the wires and deposited on the leaves below.

In the leaves of peach trees attacked by *Xanthomonas pruni*, we see the swelling of one or two layers of cells surrounding the spots. These cells become turgid, thin walled, and meristematic. When their middle lamellae are dissolved, a gap between healthy and necrotic tissues is produced. The swelling takes place mainly in the cells of palisade and spongy parenchyma. These cells become round, ovoid, long ellipsoidal or retort-like in shape. They serve to cut off the necrotic area from the healthy tissues, and this tissue gradually shrivels, dies, and sloughs off. In this way, the healthy tissues are protected from the damage, caused possibly by the toxins of the pathogen or the products of the dying lesions (Fig. 10).

Perforation of spots in the leaves of *Prunus amygdalus* takes place as a result of the attack of *Cladosporium* (*Clasterosporium*) *carpophilum* (Samuel, 1927). According to Samuel, when leaves form the abscission cells, they must be young and active, and well supplied with water.



FIG. 10. Abscission cells produced around the necrotic area in peach leaf (variety DenJuro) induced by *Xanthomonas pruni*: (p) normal palisade tissue, (t) abscission cells, (v) normal parenchyma cells in the vascular bundle. (From Akai, 1951.)

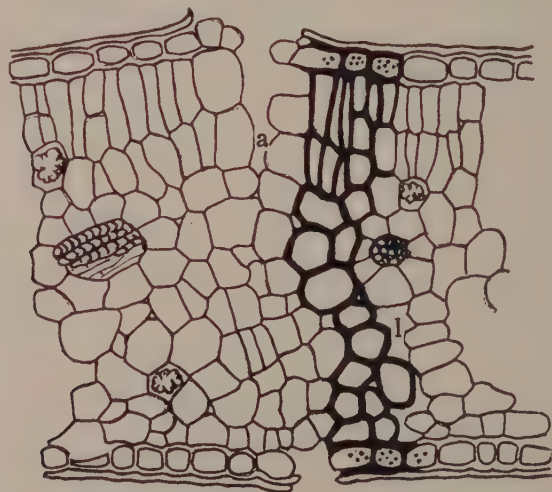


FIG. 11. Perforation of the diseased spot produced on leaf of *Prunus lauro-cerasus* infected by *Clasterosporium carpophilum*. Lignified cells (l) appear at the periphery of diseased area. The abscission layer (a) is formed outside of the lignified cells. (After Samuel, 1927.)

Therefore, the infected tissues in young leaves are invariably abscised. If leaves are old or moisture relations unfavorable, abscission cells are not formed, and the infected tissues do not fall out. In this case the meristem cells become suberized. Thus cutting off of the infected tissue may be caused by a barrier of wound cork, which also serves to check further extension of the fungus into the leaf. Moreover, cells swell in a narrow zone at some distance from the margin of the invaded area and form an abscission layer. Suberization of the walls of cells along the abscission line occurs soon after the cuticle ruptures. Subsequent divisions of the meristematic layer result in the formation of a layer of brick-shaped cells that become suberized and slightly lignified (Fig. 11). Where abscission does not occur, the initial changes are similar, but in the later stages the walls of the cells on the inner side of the occluded zone lignify and the walls of the meristematically formed cells suberize. Outside the abscission cell layer similar cork layers are formed in the healthy part of peach leaves around the spot of *Xanthomonas pruni*. This cicatricial layer may perfectly cut off the tissues from the external world, preventing excessive evaporation from the thin walled abscission cells and keeping back the injurious effect of the secondary invasion of even weakly pathogenic fungi.

On the twigs of cherry (*Prunus yedoensis*) affected by witches'-broom, small sized leaves are formed. In spring the ascospores of the causal fungus, *Taphrina cerasi*, are produced on the lower surface of these leaves. This sorus develops in a limited, localized portion of a leaf. Even in such diseased leaves, the lesion sloughs out, producing abscission cells around the area, after the ascospores mature. When the upper epidermal cells begin to collapse, the abscission cells are formed about the margin of the lesions in the same manner as in lesions of peach leaves, even if the functioning of the mesophyll and lower epidermal cells continues. Consequently, it may be assumed that the formation of abscission cells is related to the necrosis of cells, and a hormone-like substance may be considered. On the other hand, *Tranzschelia pruni spinosae* (rust fungus of peach and plum trees) possesses no such activity and, therefore, does not result in any delimiting area on leaves.

c. *Tylosis and Gummous Deposition.* Tylosis is sometimes found in the vessels of the invaded portion of plants. This tylosis may be formed by the stimulation of metabolic products of the fungus. However, literature regarding the fundamental cause of tylosis formation is contradictory. Haberlandt (1923) considered that the decomposition products of injured cells may play a decisive role in causing tyloses to form. This theory is based on the fact that tyloses are often found beneath the surface of amputated branches or adjacent to wounded areas. Klein



(1923) supports a different interpretation. He considered the presence of air within the exposed vessels to be the chief cause of tylosis formation. However, judging from Powers' observation (1954), tyloses and gums in the vessels of diseased stems are caused primarily by toxic effects of decomposition products of invaded cells. These toxic substances are not systemic in nature, but affect primarily a restricted region in the xylem. Moreover, he considered that these toxic substances are not necessarily products of fungal metabolism since cell decomposition products alone induce a similar reaction. He observed that a severe wilting of tobacco plants, with tylosis and gum formation, developed when excised healthy plants were placed in extracts of either healthy or black shank affected plant tissues. On the contrary, Bazzigher (1957) considered tylosis formation in the diseased portion of chestnut trees invaded by *Endothia parasitica* to be attributable to the stimulus of diaporthin, a metabolite of this fungus.

Tylosis clogging in vessels impedes the flow of water. Such mechanical blocking of conducting elements by tyloses is held as the chief factor responsible for wilting and drying of leaves on infected chestnut trees (Bramble, 1938). In oak trees, infected by *Chalara quercina* (*Endoconidiophora fagacearum*), extensive plugging of the xylem vessels with tyloses and gums precedes the foliage wilt. Tyloses are formed in the large vessels of the spring wood, especially of the last annual ring, but less so in the small vessels of the summer wood (Struckmeyer *et al.*, 1954). In the xylem of diseased sweet potatoes tylosis and vascular discoloration usually occur in advance of the invading mycelium of the wilt fungus, *Fusarium oxysporum* f. *batatas* (Watanabe, 1939; McClure, 1950). In this case the tylosis-clogged vessels may serve to prevent the invasion of the pathogen. Tyloses have cellulose walls, formed from adjacent living cells by extrusion through half-bordered pits. Often they are so numerous and large that they become closely packed in the vessel lumen, losing their original spherical shape. After staining by Cartwright's method, the walls of most of the tyloses are blue, except those nearest to the pathogen, which are stained red. In this latter region a substance which appears to be wound gum accumulates in the interstices between tyloses, and between tyloses and their enclosing vessel wall. Penetration of the tylosis blockage or rupture of tyloses by the wilt fungus has not been observed. Hyphae pass through vessel element apertures and through unobstructed pits, but do not directly penetrate cell walls or pits which are covered with wound gum (McClure, 1950).

There seem to be two types of gum materials secreted by plant tissues (Yoshii and Kawamura, 1947): one is the gum which appears on stone fruits, and the other a wound gum which shows a lignin-like color

reaction. The former gum appears most often on fruits, branches, or trunks of stone fruit trees, and is also associated with injury from insects or mechanical sources. Valsa disease of peach always produces gummosis if the trees are vigorous. The gum is mainly composed of pentosans as shown by the pectin-like reaction, and is produced by the liquefaction of woody membranes. On the other hand, wound gum deposits occur in the injured portion, where the substance fills the cell lumen and sometimes permeates the cell walls, especially on the abnormally swollen walls (Yoshii, 1948).

Gum deposition along the border of diseased lesions often serves as a protective demarcation and constitutes a type of mechanical resistance. Surrounding the necrotic lesion on the leaves of Unshu orange produced by *Phyllosticta*, wound gum deposits demarcate effectively the healthy tissues from the diseased lesion, by causing a marked constriction of the necrotic lesions (Yoshii, 1949). In the stems of cherry affected by canker disease, the causal fungus of which is *Valsa japonica*, similar gum-like deposits are formed in wood vessels. Gum gradually replaces the starch and other contents in the medullary ray cells, wood parenchyma, and the wood vessels are slowly plugged up by the deposits of the gum (Hemmi, 1916).

In the silver leaf disease caused by *Stereum purpureum*, under conditions favorable for gum formation, the wood of the host produces so much gum in advance of the fungus in a relatively short time, that the fungus becomes completely enclosed by an impassable gum barrier. Within this barrier, the fungus may continue to live for a considerable time, but eventually it dies. These gum barriers may correspond to the protective wood (Frank, 1895), as developed by the deposition of gum and browning of cell walls. They usually develop within an inch of the wound and require at least two months for their completion (Brooks and Brenchley, 1931). Hesler (1916) has found a similar gum barrier in the diseased part of apple twig, infected by *Physalospora cydoniae* showing a brown deposit in wood fibers and wood parenchyma cells (Fig. 12).

In noninoculated cotyledons of "Proso," a scab-resistant variety of cucumber, mechanical damage by scratching induced a wound reaction. This was revealed by the secretion of a granulated yellow substance that almost filled the intercellular spaces between healthy cells in the neighborhood of the damaged zone. In addition, the walls of some of these cells become yellowish in color and no longer stained with zinc chloride-iodine. The yellow substance stains with ruthenium red, and reacts positively to the lignin test with phloroglucin-hydrochloric acid. In "Proso," 3-5 days after inoculation with *Cladosporium cucumerinum*, the mycelium seems to stimulate secretion of the yellow substance so that it fills

the interior of the healthy cells as well as the intercellular spaces in an almost uninterrupted zone around the wound. The contents of some of the cells in this zone contain a yellow granular substance, whereas the cell walls are yellow in color and slightly swollen. Within this zone, hyphae are rarely found and beyond it they are entirely absent. Evidently this zone acts as a barrier against further spreading of the causal fungus. Probably the formation of the yellow granular substance plays an important role in this respect (El-Din Fouad, 1956).



FIG. 12. Gum barrier in the apple twig infected by *Physalospora cydoniae*. Mycelium is shown in the xylem ducts. (After Hesler, 1916.)

In the wilt disease of sweet potatoes, wound gum is also produced. Wound gum, which is golden brown but stains deeply with safranin, is often found in hemispherical masses which protrude into the lumen of the invaded vessel. These gum deposits are usually located on half-bordered pits, and apparently secreted through the pits by the adjacent

living cells. Bordered pits sometimes bear gum deposits, but in every case a living tylosis is contiguous with the other face of the pit (McClure, 1950). Thus, the wood gum formed in half-bordered pits in the vicinity of the pathogen may act, physically or chemically, as a barrier which prevents hyphal penetration of the adjacent living cells. Consequently, wound gum may play a part in preventing both the penetration of the pathogen into tyloses and its intrusion between the vessel wall and tylosis wall.

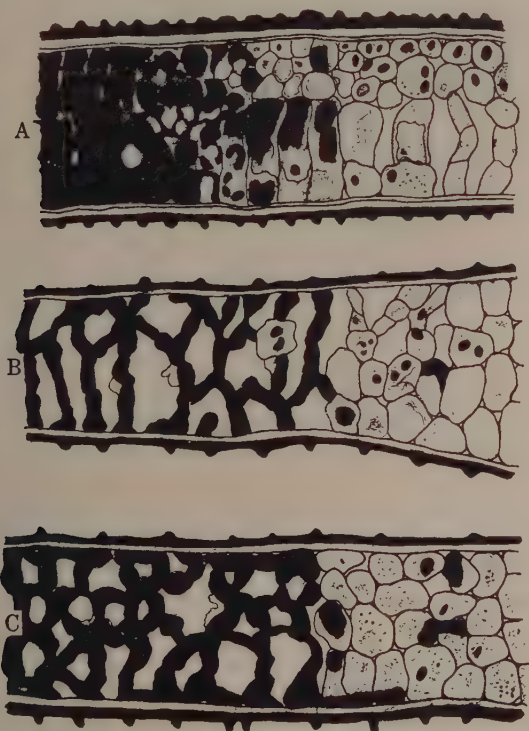


FIG. 13. The brown change of cells in blast diseased lesion of rice leaves: (A) Kan-Non-Sen highly resistant, (B) Gin-Nen resistant, (C) Kokuryo no Miyako susceptible. (After Kawamura and Ono, 1948.)

In varieties of rice resistant to blast disease or *Helminthosporium* leaf spot, similar deposits are formed in the intercellular spaces that aid in restricting the fungus to the area of primary invasion. These deposits are found to be highly developed in Shoemed rice (resistant to helminthosporiose), but they also have been found to some extent in susceptible



varieties (Tullis, 1935). In varieties of rice resistant to helminthosporiose and blast disease, even after infection by the causal fungus, necrotic cells are filled with brown wound gum-like substances, but do not show any shrinkage. The failure of cells to shrink may play an important part as a defense reaction together with the antifungal substances extruded from cells (Kawamura and Ono, 1948; Yoshii, 1957) (Fig. 13, Table V).

## 2. Callus-like Swelling and Callosity

a. *Swelling of the cell wall.* In the infected host, contact with hyphae sometimes results in swelling of cell walls. This is observed frequently in cuticular infection. Before the entry of *Botrytis cinerea* into pea leaf cells, swelling at the point of penetration is found in the subcuticular layer of cell walls, without causing any change in the cuticle (Blackman and Welsford, 1916). This swelling seems only a softening of the wall, because the fungus is soon able to penetrate it. The actual penetration is effected by pressure exerted on the underlying tissues, accompanied by the development of a fine peg-like growth from the appressorium, which is firmly pressed against the leaf surface. However, some consider that the penetration of cuticular barrier appears to be effected by chemical rather than by mechanical action (Woodward, 1927).

In at least one case, the thickened wall becomes lignified and acts as a principal factor in resistance to penetration. This barrier may exclude the fungus effectively. When the living leaves of tomatoes were artificially inoculated with conidia of *Piricularia oryzae*, the rice blast fungus, the cells resist penetration. At first, the hyphae form appressoria on the epidermal wall, which reacts by swelling at the contact portion of the appressoria. This swollen portion shows a lignin-like reaction, and is not transparent to light under crossed Nicol prisms. The fungus seems to have difficulty in penetrating such a cell (Fig. 14) (Yoshii, 1948). These abnormal thickenings of cell walls are found in oats attacked by *Helminthosporium avenae*, and in flax attacked by *Fusarium lini* (Tisdale, 1917). The latter is attributed to the formation of suberin. Subepidermal cell walls of corn roots also become thickened prior to infection by *Helicobasidium mompa* (Fig. 15) (Ito, 1952). These thickened walls are lignified (perhaps permeated with wound gum substance), and the hyphae are prevented from penetrating the thickened wall.

*Cladosporium cucumerinum* seemed to enter equally well both the resistant and susceptible varieties of cucumber. However, the progress of the fungus within the tissue was arrested by the host-parasite interaction which is associated with cell wall thickening and cell necrosis (Pierson and Walker, 1954). This is the mechanism which confines the

TABLE V  
PRIMARY REACTION OF CELLS IN RICE LEAVES AT THE POINT OF ENTRY OF BLAST FUNGUS AND THEIR SUSCEPTIBILITY<sup>a</sup>

Variety	Primary reaction in cells	Resin-like deposit	Constriction of necrotic cells	Size of spots	Number of infection	Susceptibility
Kan Non Sen	Rapid	Abundant	None	Minute	Numerous	Highly resistant
Gin Nen	Rapid	Considerable	Markedly	Large	Considerable	Resistant
Ko Sen	Slow	Small	Markedly	Middle	A few	Resistant
Kameji	Slow	Considerable	Markedly	Large	Considerable	Resistant
Kaiyo Shinriki	Slow	Considerable	Considerable	Large	Considerable	Susceptible
Wase Asahi	[Slow	Small	Markedly	Middle	A few	Susceptible

<sup>a</sup> After Kawamura and Ono, 1948.

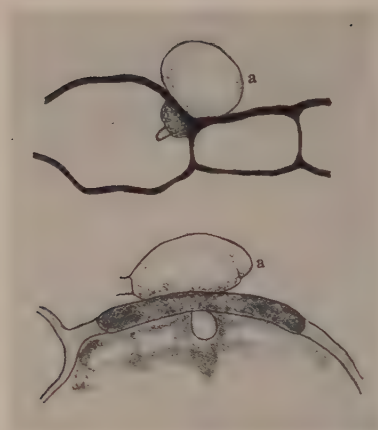


FIG. 14. Mode of infection of *Piricularia oryzae* artificially inoculated on the living tomato leaf using hyphae: (a) appressorium. (After Yoshii, 1948.)



FIG. 15. Young root cells of corn infected by *Helicobasidium mompa* showing the thickened wall of subepidermal cells. Hyphae are constricted more or less when they pass through the wall. (After Ito, 1952.)

disease to a relatively small number of host cells and prevents the formation of large lesions.

b. *Callosities*. In some cases, when hyphae start to penetrate cell walls, a slight protuberance is formed on the opposite wall. This protuberance elongates at right angles to the wall, directly facing the advancing hyphae (Fig. 16). Sheaths enclosing the invading hyphae were probably described first by De Bary (1863), and these have been called callosities (Young, 1926).

The callus (callosity) formed on epidermal walls usually obstructs the invasion of *Olpidium viciae* into the cell. The effectiveness of callus

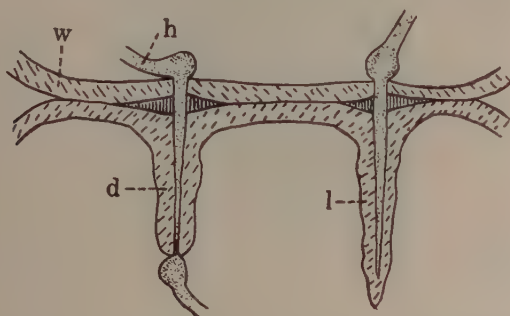


FIG. 16. The penetration of cell walls by penetrating hyphae and ligni-tubers formed about them: (d) ligni-tuber through which the hypha has passed, (h) hypha, (l) ligni-tuber through which the hypha has not passed, (w) cell wall. (After Fellows, 1928.)



FIG. 17. Infection mode and callosity formation in cells of the rush (*Juncus effusus* var. *decipiens*) invaded by *Leptosphaeria juncina*. (After Ikata and Yoshida, 1940.)



varies according to the plant species. In appropriate hosts, such as *Vicia unijuga*, *V. faba*, and *Pisum sativum*, this parasite is able to enter the cell through the callus. In other hosts such as *Impatiens*, *Taraxacum*, *Oenothera*, etc., callus is more effective in defense. In some nonsusceptible plants (*Chrysanthemum*, *Lactuca*, *Dahlia*, etc.), the callus actually blocks out all infectious individuals (Kusano, 1936).

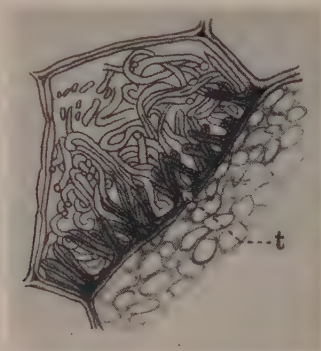


FIG. 18. Mycorrhizal cell of rhizome of *Gastrodia elata* showing the formation of numerous tubular sheath (t). (After Kusano, 1911.)

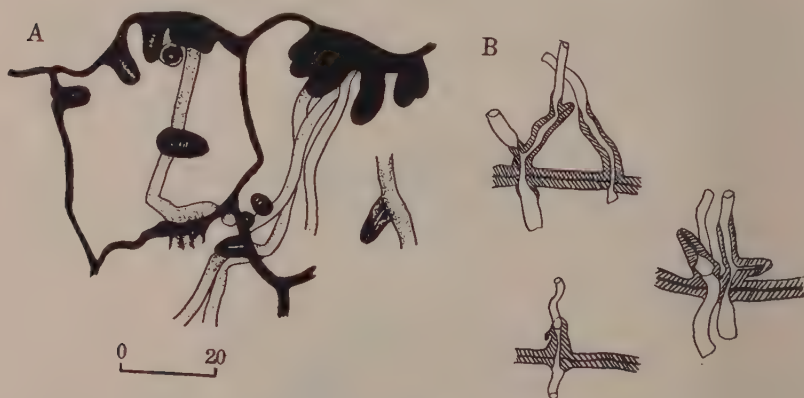


FIG. 19. Tubular sheath produced on the wall of cork cells, of *Gastrodia callosa* and on the hyphae (A) and of *Galeola hydra* (B). (After Burgeff, 1932.)

In straws of Japanese rush (*Juncus effusus* var. *decipiens*), the penetrating hyphae of *Leptosphaeria juncina* form callosities in epidermal cells. The callosity is formed before the hyphae pass through the epidermal cell wall. Callosities are generally formed most vigorously in young stems of plants (Fig. 17) (Ikata and Yoshida, 1940).

In the mycorrhizal cells of tubers of *Gastrodia elata*, a tubular sheath forms on the lignified or unlignified walls. In some cases (Fig. 18) it occurs as an aggregate (Kusano, 1911). Burgeff (1932) also found a tubular sheath in the mycorrhizal cells of *Galeola hydra* and *Gastrodia callosa* (Fig. 19).

Evidence has been cited concerning the wart-like protuberances (sheaths) that form on the inner wall opposite the penetration point as if to impede penetration of the haustorium of powdery mildews, downy mildews, and rusts.

Many investigators have stated that callosities or sheaths are produced mainly from the cell walls. Smith (1900) referred to it as a cellulose collar, formed by the protruding cell wall, and Corner (1935)

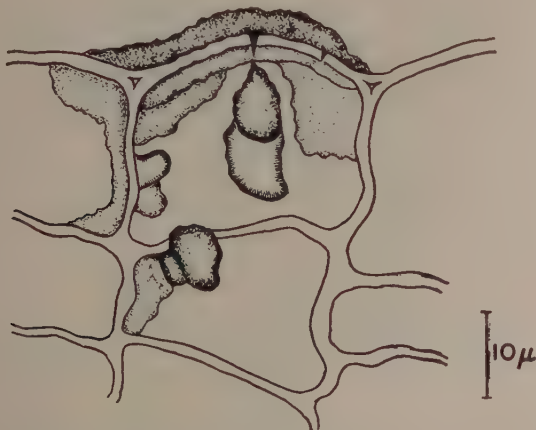


FIG. 20. Callosity (callosity-like body) produced on the epidermal cell wall of stem of the sweet potato seedling by needle pricking. (After Ito, 1949.)

considered it a swelling of wall and called it a "papilla." Kusano (1936), however, has a different opinion: that the sheath of the haustorium is formed directly by the accumulation and aggregation of cytoplasm and is not a deformation of the wall. Aronescu (1934) pointed out that the collar-shaped basal mass surrounding haustoria seems to be composed of materials deposited by the cytoplasm at the same time that the haustorium advances into cells. Ito (1949), however, concluded that at least the callosities found on the cork cells are produced directly from the wall.

The callosities are induced not only by the fungus hyphae, but also by mechanical injuries. Some examples were shown by Ito (1949) in which callosities are formed by pricking the epidermal cell walls of

sweet potato stem with a sterile needle (Fig. 20). Some consider that the callosity functions to heal the wound caused by an invader and not to check the fungus invasion (Kusano, 1939). Accordingly, there is no close connection between the formation of the callosity and the resistance of the plant, a matter possibly recognized in some cases (Iwata, 1940).

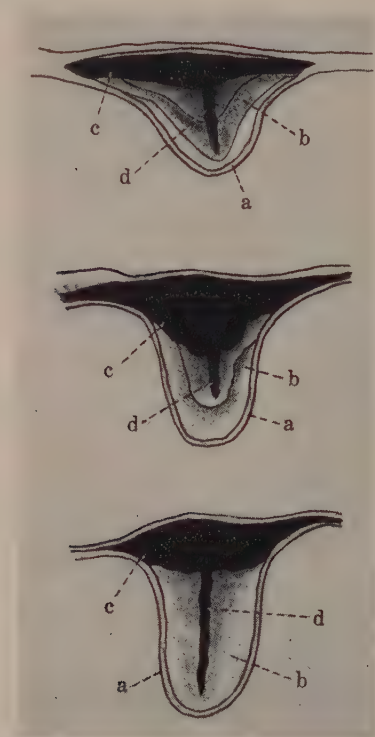


FIG. 21. The structure of the callosity produced on phellem cell wall of sweet potato stem (stained with Sudan III and methyl green): (a) outer layer staining light reddish (b) middle layer staining light purplish green (c) basal part (deep purplish green) (d) piercing filament. (After Ito, 1949.)

The callosity on the phellem consists of about three layers (Ito, 1949). The outer membrane, middle layer and basal part are seen clearly together with a piercing filament in the central portion (Fig. 21). In many cases, the sheath is formed in young vigorous cells (Chu, 1935; Akai, 1950), and also when haustoria become debilitated (Rice, 1927). Haustoria formed in cells of *Brassica* infected by *Albugo candida*, appeared to

be covered with a wall when they became debilitated (Akai, 1942). Haustoria of stripe rust (*Puccinia glumarum*) which are dead or in an early stage of degeneration, are also formed with a heavy sheath (Allen, 1928). Haustoria of *Marasmiella hyalospora*, a rust fungus of *Acacia confusa*, become covered after maturity. In such a case they are sometimes dead (Hirane, 1940).

Almost all hyphae or haustoria are able to grow out through their sheath and enter the lumen of cells and when they do, they immediately increase to normal diameter again. However, some callosities are impossible to penetrate. Ito (1949) observed no hypha piercing through a callosity formed in the wall of phellem cell.



FIG. 22. Cellulose cushion, in which the hypha of *Ustilago zeae* is enclosed. (After Guttenberg, 1905.)

The sheaths may be composed of cellulose (rust and downy mildew), but some are made up of callose (Mangin, 1896). Afterward, in some cases, a gum-like substance (wound gum) permeates them. Protuberances formed on the walls of cells in wheat roots infected by *Ophiobolus graminis* contain no trace of callose, but rather are composed chiefly of lignin (Fellows, 1928; Robertson, 1932). Therefore, in place of the terms callosity or callus used by Stevens (1922) and Young (1926), Fellows has (1928) suggested the name "lignituber."

c. *Cellulosic covering of hyphae*. Often the cellulose that envelopes the hyphae extends out in cells as a defensive reaction of the host. In



galls of corn smut, Guttenberg (1905) found a cellulose cushion, in which the hyphae of *Ustilago zeae* were enclosed (Fig. 22). This may be a defense of the host plant against hyphal intrusion. In addition to the tubular sheath, hyphal branches are covered in the mycorrhizal cells of *Gastrodia callosa* (Burgeff, 1932).

### B. Defense Originating from Cell Reactions

Some defensive cell reactions are of cytoplasmic origin. As a rule, these reactions are unable to prevent infection, thus keeping the parasite at a distance. Infection, therefore, occurs in most cases, but afterwards the antiparasitic defense reaction is evoked and this limits the pathogen to a certain tissue by a necrogenous cell reaction. Consequently, these defense reactions prevent the pathogen from progressing from its initial point of infection to a generalized infection. Thus, the host is protected from suffering serious injury.

As Gäumann (1950) has noted, two aspects of this phenomenon can be considered: (1) plasmatic defense reactions resulting from biochemical functioning of living cells and (2) the necrogenous defense reaction resulting from death of cells at the infection point.

#### 1. Plasmatic Defense Reaction

Plasmatic defense is the response of the living plasma of cells against the pathogen. As Gäumann (1950) illustrated, plasmatic defense is observed in the case of weakly pathogenic organisms which remain in contact for a long time with their host cell and evoke a chronic disease.

The relation of the endophytic nitrogen-fixing *Bacterium radicola* in the root nodules of leguminous plants, follows the three possible lines depending on the balance of forces between host and parasite: either the host remains uninjured by the parasite, the parasite overcomes the host, or they are about evenly balanced (Schaeede, 1932; Gäumann, 1950).

In mycorrhiza, the host-parasite relationship is similar in character to that prevailing between leguminous plants and their nodule bacteria. This is true in both the endophytic and ectophytic mycorrhizae. The plasmatic defense reaction operates to induce a weakening, localization, and elimination of the endophyte.

Typical mycorrhizal formation results only in the case where fungus and host cells are evenly balanced. They remain in temporary equilibrium. Sometimes, however, the host cytoplasm is consumed by the parasite. Conversely, more centrally situated digestion cells deteriorate the parasite.

The tuberous rhizome of *Gastrodia elata* forms an endotrophic my-

corrhiza with the mycelial strands of *Armillaria mellea* (Kusano, 1911). In the outer region of mycorrhizal cell layers the cytoplasm invests the hyphal clump and the nucleus is stretched, often so much as to be divided into two portions (Fig. 23). When the clump becomes larger, the protoplast disappears entirely. In the inner region (digestion cells), the cytoplasm increases in amount and acquires a granular and dense consistency, while the nucleus undergoes hypertrophy, hyperchromatophily, and various deformations by constriction. Prominent bodies appear in the cell which comprise both secretions and excretions of the endophyte. Light yellowish oil-drop-like globules and similar sized vesicles within a hyaline membrane become visible in the cytoplasm.

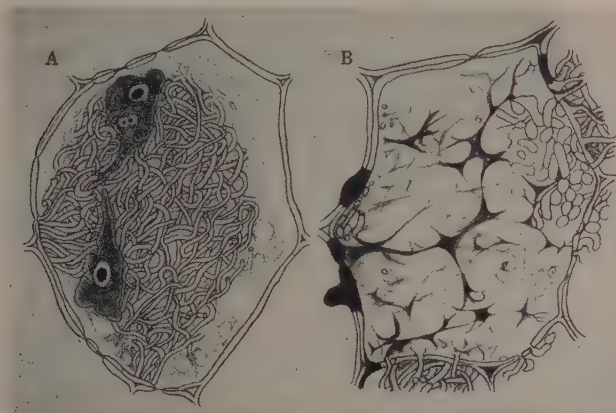


FIG. 23. Mycorrhizal cells of the outer region in rhizome of *Gastrodia elata*: (A) Mycorrhizal cell in the outer region with hyphal clump surrounded by the cytoplasm, nucleus is stretched into two parts. (B) Hyphae in the process of self-disorganization. (After Kusano, 1911.)

These contain yellowish granules. They are consumed later by the host. In these digestion cells an accumulation of very fine granules is observed around the hyphae previous to their disintegration. Probably this phenomenon is connected with the digestive action of the host (Fig. 24). In the digestion cells of *Gastrodia javanica*, invaded hyphae of the mycorrhizal fungus swell in a ptyosome, which is digested afterwards (Fig. 25) (Burgeff, 1932).

In the acutely infectious diseases, plasmatic defense reactions have the same tendencies to weaken, localize, and eliminate the intruding parasite as have been described above. However, the plasmatic defense reaction does not generally function with high efficiency. As a rule,

*Olpidium viciae* most readily infects plants the cells of which are attractive to swarm spores and which offer a suitable site for the swarm spores to encyst. On the surface of *Oenothera*, *Taraxacum*, *Impatiens*, and *Physalis*, swarm spores of *Olpidium viciae* are attracted to their tissues, and can easily invade them. However, a defensive reaction inside the cell may interfere with the development of the parasite. Death of the parasite occurs in the plant cell. Thus, the defensive action is not always of the same strength (Kusano, 1936).



FIG. 24. Disintegration of hyphae in the mycorrhizal cells of rhizome of *Gastrodia elata*: Disintegration of hyphal branches into small granular bodies (a), and into excretion bodies (b) at the end of activity of the mycorrhizal cell. (After Kusano, 1911.)



FIG. 25. Ptyosome (p) formation in mycorrhizal cell of *Gastrodia javanica*: (d) digesting ptyosome, (n) nucleus of host cell. (After Burgeff, 1932.)

Latent infection of anthracnose fungi in healthy citrus leaves or fruits may be due to a plasmatic defense of cells, where the fungi and hosts may be evenly balanced, though the details in this phenomenon are unknown (Tokunaga and Yokohama, 1955).

## 2. Necrogenous Defense Reaction

The defensive potentialities of plants are not destroyed with the failure of the normergic plasmatic defense reactions. In many infectious plant diseases, another type of reaction now comes into operation. This is the necrogenous or aborting defense reaction. Postinfectionally, this reaction results in the necrosis of cells. As a result the invading parasite is left isolated or is retarded in its growth by an interruption in the nutrient supply.

Necrogenous defense is demonstrated clearly in the case of the wart disease of certain potato varieties due to the infection of *Synchytrium endobioticum*. In immune varieties, the parasite and host are incompatible; they react antagonistically to one another. In certain varieties, e.g., in Ackersegen, the reaction results in death of the infected epidermal cells. Death of these cells is rapid and their protoplasm is converted into a peculiar brown mass (gummosis). They lose their turgor and sometimes are compressed by neighboring cells (Köhler, 1928, 1931; Gäumann, 1950).

As noted above in the scab-resistant "Proso" variety of cucumber, the situation is similar to that in the wart-immune potato varieties. The presence of the mycelium of *Cladosporium cucumerinum* seems to stimulate the secretion of a yellow substance that diffuses from the parasitized cell. The necrogenous reaction is then set up in the noninfected neighboring cells (El-Din Fouad, 1956). In consequence, the nutrient supply in the focus of infection is cut off; the parasite is prevented from maturing and reproducing itself (subinfection) so that the infection chain is broken and secondary infection does not take place.

Kannon Sen (indica type of rice plant) is highly resistant to blast disease. As has been shown in Table V, the infection occurs comparatively easily, but the reaction of cells leads to their necrosis. Then the necrogenous reactions are set up at the focus of infection. Thus, the cells die rapidly, showing abundant minute spots on leaves. The parasitized cells become filled with a gummy deposit. Consequently further growth of the parasite is interrupted and it is finally destroyed.

In general, some varieties of Japanese rice (*Oryza sativa*), highly susceptible to *Piricularia oryzae*, do not show any visible plasmatic change on infection; whereas, the epidermal cells of leaves in *Oryza minuta* and *O. latifolia* react antagonistically to the fungus invasion. Hence, the abortive reaction in the infected cells is evoked. This shows



that the intruded hypha was enveloped within resin-like materials, and its growth was completely interrupted. (Kawamura, 1940) (Fig. 26).

Browning of cell contents is classified into several types according to the mode in which the brown granular bodies are deposited (Kawamura and Ono, 1948). Cellular browning occurs with no shrinkage and involves a type of resistance (Fig. 13). However, according to the observations of Takahashi (1956), uncolored or slightly colored granular changes (slight yellow to slight yellowish brown) are better related to resistance. The penetrating hyphae of *Piricularia oryzae* do not grow in the uncol-

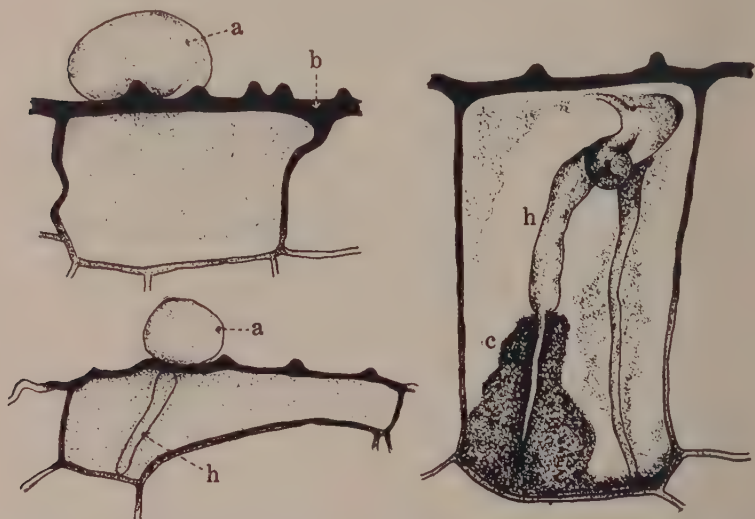


FIG. 26. Epidermal cell of *Oryza minuta* in which the intruded hypha of *Piricularia oryzae* is enveloped within resin-like deposit (c): (a) appressorium, (b) brown changed membrane, (h) hypha. (After Kawamura, 1940.)

ored or slightly colored cells of the rice leaf sheath, or if they do grow, the growth is scant. When dark brown granules are produced in cells they reveal more or less where penetrating hyphae have grown. When these brown granules accumulate in the parasitized cells, the whole cell becomes colored dark or black-brown. The hyphae usually do not grow out of such cells. In some cases, however, a weak growth of hyphae is recognized in the neighboring unchanged cells.

When invaded by *Phytophthora infestans*, cells of highly resistant potato varieties degenerate. Five phases of this degeneration process can be distinguished (Fig. 27) (Tomiya, 1956). Fungal penetration of highly resistant varieties accelerates the appearance of protoplasmic

strands and the migration of the nucleus toward the infection point. Thus, the rhythmic movement of the nucleus around the infected part is evoked in the early stage of the infection (phase 1). Then the movement of the granules becomes that of Brownian motion (phase 2) and finally the death of cells takes place (phase 3) accompanied by deep browning of cell. At this time (phases 4 and 5), deposition of polyphenols and excretion of other compounds may take place which brings about the death of the invaded hyphae.

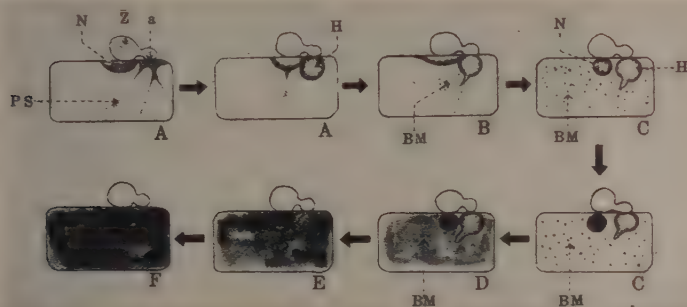


FIG. 27. Diagrammatic illustration of degeneration process in the cells of highly resistant potato variety infected by *Phytophthora infestans*: (BM) Brownian motion, (H) infecting hypha, (N) nucleus, (PS) protoplasmic strands, (Z) zoospore.

Phase 1: The infection hyphae begin to invade the host cell. No changes are observed except the migration of nuclei toward the infected part, and the occurrence of active protoplasmic strands or protoplasmic streaming around the infected center (a) (A).

Phase 2: A number of small granules, rod shaped or spherical in Brownian motion, appear around the hyphae, and gradually increase in number. The death of cells has not yet taken place in this phase (B-C).

Phase 3: The host cell dies, and the cell content turns yellowish, while the small granules continue the Brownian motion (D).

Phase 4: The small granules stop their motion, and the cell content turns pale brown (E).

Phase 5: The color of the cell content becomes deep brown and finally turns blackish (F). (After Tomiyama, 1956.)

## REFERENCES

- Akai, S. 1942. On the anatomical studies of the hypertrophied stems of rape infected by the conidial stage of white rust fungus. *Botan. and Zool. Tokyo* **10**: 631-633.
- Akai, S. 1950. Studies on the pathological anatomy of fungus galls of plants. *Mem. Coll. Agr. Kyoto Univ.* **58**: 1-60.
- Akai, S. 1951. On the anatomy of necrotic lesions on leaves and stems of plants affected by pathogenic fungi. *Mem. Coll. Agr. Kyoto Univ.* **61**: 1-30.

- Allen, R. F. 1928. A cytological study of *Puccinia glumarum* on *Bromus marginatus* and *Triticum vulgare*. *J. Agr. Research* **36**: 487-513.
- Aoki, K. 1941. On fungi concealed in the lenticels of healthy mulberry-trees. *Bull. Imp. Sericult. Expt. Sta. Japan* **10**: 229-281.
- Aoki, K. 1945. Ecological studies of mulberry-blight, "Dogare-disease." *Bull. Sericult. Expt. Sta. (Japan)* **12**: 245-306.
- Araki, T., Y. Yamazaki, and N. Suzuki. 1957. Studies on the violet root rot of sweet potatoes caused by *Helicobasidium mompa* Tanaka. IV. Production of itaconic acid by *Helicobasidium mompa*. *Bull. Natl. Inst. Agr. Sci. (Japan) Ser. C* **8**: 53-59.
- Aronescu, A. 1934. *Diplocarpon rosae*: from spore germination to haustorium formation. *Bull. Torrey Botan. Club* **61**: 291-329.
- Artschwager, E. 1927. Wound periderm formation in the potato as affected by temperature and humidity. *J. Agr. Research* **35**: 995-1000.
- Bach, W. J., and F. A. Wolf. 1928. The isolation of the fungus that causes citrus melanose and the pathological anatomy of the host. *J. Agr. Research* **37**: 243-252.
- Bazzigher, G. 1957. Ueber Anfälligkeit und Resistenz verschiedener Wirte von *Endothia parasitica*. *Phytopathol. Z.* **30**: 17-30.
- Blackman, V. H., and E. J. Welsford. 1916. Studies in the physiology of parasitism. II. Infection by *Botrytis cinerea*. *Ann. Botany (London)* **30**: 389-398.
- Bramble, W. C. 1936. Reaction of chestnut bark to invasion by *Endothia parasitica*. *Am. J. Botany* **23**: 89-94.
- Bramble, W. C. 1938. Effect of *Endothia parasitica* on conduction. *Am. J. Botany* **25**: 61-65.
- Brooks, F. T., and G. H. Brenchley. 1931. Silver-leaf disease. VI. *J. Pomol. Hort. Sci.* **9**: 1-29.
- Burgeff, H. 1932. "Saprophytismus und Symbiose. Studien an tropischen Orchideen." Fischer, Jena.
- Caldwell, R. M., and G. M. Stone. 1936. Relation of stomatal function of wheat to invasion and infection by leaf rust (*Puccinia triticina*). *J. Agr. Research* **52**: 917-932.
- Chu, H. T. 1935. Notes on the penetration phenomena and haustorium formation in *Peronospora brassicae* Gäum. *Ann. Phytopathol. Soc. Japan* **5**: 150-157.
- Conant, G. H. 1927. Histological studies of resistance in tobacco to *Thielavia basicola*. *Am. J. Botany* **14**: 457-480.
- Cormack, M. W. 1937. *Cylindrocarpon ehrenbergi* Wr. and other species, as root parasites of alfalfa and sweet clover in Alberta. *Can. J. Research Ser. C* **15**: 403-424.
- Corner, E. J. H. 1935. Observations on resistance to powdery mildew. *New Phytologist* **34**: 180-200.
- Cunningham, H. S. 1928. A study of the histologic changes induced in leaves by certain leaf-spotting fungi. *Phytopathology* **18**: 717-751.
- Darling, H. M. 1937. A study of scab resistance in the potato. *J. Agr. Research* **54**: 305-317.
- De Bary, A. 1863. Recherches sur le développement de quelques champignons parasites. *Ann. sci. nat. Botan. et biol. végétale* **20**: 5-148.
- El-Din Fouad, M. K. 1956. Studies on genetic and on chemically induced resistance of cucumber tissues to *Cladosporium cucumerinum* (Ell. et Arth.). *Mededel. Landbouwhogeschool Wageningen* **56**: 1-51.

- Fellows, H. 1928. Some chemical and morphological phenomena attending infection of the wheat plant by *Ophiobolus graminis*. *J. Agr. Research* **37**: 647-661.
- Frank, A. 1895. "Die Krankheiten der Pflanzen." 2nd ed. E. Trewendt, Breslau.
- Gäumann, E. 1950. "Principles of Plant Infection" (English ed. by W. B. Brierley), Crosby Lockwood, London.
- Guttenberg, H. R. 1905. "Beiträge zur physiologischen Anatomie der Pilzgallen." W. Engelmann, Leipzig. pp. 1-70.
- Haberlandt, G. 1923. Wundhormone als Erreger von Zellteilungen. *Beitr. allgem. Botan.* **2**: 46.
- Hart, H. 1929. Relation of stomatal behavior to stem-rust resistance in wheat. *J. Agr. Research* **39**: 929-948.
- Hashioka, Y. 1950. Studies on the mechanism of prevalence of the rice blast disease in the tropics. *Taiwan Agr. Research Inst. Tech. Bull.* **8**: 1-237.
- Hawkins, L. A., and R. B. Harvey. 1919. Physiological study of the parasitism of *Pythium debaryanum* Hesse on the potato tuber. *J. Agr. Research* **18**: 275-297.
- Hemmi, T. 1916. On a new canker disease of *Prunus yedoensis*, *P. mume* and other species caused by *Valsa japonica* Miyabe et Hemmi sp. n. *J. Coll. Agr. Tôhoku Imp. Univ.* **7**: 257-319.
- Hesler, L. R. 1916. Black rot, leaf spots, and canker of Pomaceous fruits. *Cornell Univ. Agr. Expt. Sta. Bull.* **379**: 53-148.
- Hirane, S. 1940. Studies on the parasitism of the rust of *Acacia confusa* Merrill, *Maravalia hyalospora* (Saw.) Diet. III. *Ann. Phytopathol. Soc. Japan* **10**: 171-185.
- Hursh, C. R. 1924. Morphological and physiological studies on the resistance of wheat to *Puccinia graminis tritici* Erikss, and Henn. *J. Agr. Research* **27**: 381-412.
- Ikata, S., and M. Yoshida. 1940. Studies on disease of rush I. *Okayama Prefecture Agr. Expt. Sta. Spec. Rept.* **42**: 1-47.
- Ito, K. 1949. Studies on "Murasaki-Mompa" disease caused by *Helicobasidium mompa* Tanaka. *Bull. Govt. Forestry Expt. Sta. (Japan)* **43**: 1-126.
- Ito, K. 1952. Immunity of gramineous plants to the Murasaki-mompa disease. *Agr. and Hort. Tokyo* **27**: 85-86.
- Ito, S., and M. Sakamoto. 1939. Studies on blast disease of rice plants. *Progr. Repts. 1938 Coll. Agr. Hokkaido Imp. Univ.* pp. 1-58.
- Ito, S., and S. Shimada. 1937. Studies on blast disease of rice plants, with special reference to the infection process of the causal fungus and the varietal resistance of rice plants. *Contrib. Improvement Agr., Ministry Agr. and Forestry, No.* **120**: 1-109.
- Iwata, Y. 1940. Studies on the penetration of *Peronospora aparines* (deBary) Gäum, and the relation of the epidermal cell. *Ann. Phytopathol. Soc. Japan* **10**: 203-213.
- Jahrmann, F. 1913. Ueber Heilung von Epidermiswunden. *Centr. Bakteriöl. Parasitenk. Abt. II* **37**: 564-595.
- Jones, A. P. 1931. The histogeny of potato scab. *Ann. Appl. Biol.* **18**: 313-333.
- Kawamura, E. 1940. Reaction of certain species of the genus *Oryza* to the infection of *Piricularia oryzae*. *Bull. Sci. Fac. Terkult. Kyusyu Imp. Univ. Fukuoka, Japan* **9**: 157-166.
- Kawamura, E., and K. Ono. 1948. Studies on the resistance of foreign rice plants to blast disease. *Bull. Natl. Agr. Expt. Sta. Japan* **4**: 13-22.
- Klein, G. 1923. Zur Aetiologie der Thyllen. *Z. Botan.* **15**: 417-439.



- Köhler, E. 1928. Fortgeführte Untersuchungen über den Kartoffelkrebs. II, III. *Arb. biol. Reichsanstalt Land- u. Forstwirtsch. (Berlin-Dahlem)* **15**: 135-176, 401-416.
- Köhler, E. 1931. Ueber das Verhalten von *Synchytrium endobioticum* auf anfälligen und widerstandsfähigen Kartoffelsorten. *Arb. biol. Reichsanstalt Land- u. Forstwirtsch. (Berlin-Dahlem)* **19**: 263-284.
- Kurosaki, Y. 1957. Ueber die Beziehung zwischen der Fleckengröße-Verteilung und der Widerstandsfähigkeit von Reispflanzen bei der Helminthosporiose. *Ann. Phytopathol. Soc. Japan* **22**: 251-256.
- Kusano, S. 1911. *Gastrodia elata* and its symbiotic association with *Armillaria mellea*. *J. Coll. Agr. Imp. Univ. Tokyo* **4**: 1-82.
- Kusano, S. 1936. On the parasitism of *Olpidium*. *Japan. J. Botany* **8**: 155-187.
- Kusano, S. 1939. Relation between the fungus infection and the callus-formation of the membrane. *Ann. Phytopathol. Soc. Japan* **9**: 111-112.
- Leach, J. G. 1931. Blackleg disease of potatoes in Minnesota. *Minn. Agr. Expt. Sta. Tech. Bull.* **76**: 1-36.
- Longrée, K. 1931. Untersuchungen über die Ursache des verschiedenen Verhaltens der Kartoffelsorten gegen Schorf. *Arb. biol. Reichsanstalt Land- u. Forstwirtsch. (Berlin-Dahlem)* **19**: 285-336.
- Lutman, B. F. 1945. The spread of potato scab in soil by potato plant humus. *Verмонт Agr. Expt. Sta. Bull.* **528**: 1-40.
- Mangin, L. 1896. Recherches anatomiques sur les Péronosporées. *Bull. soc. hist. nat. Autun.* **8**: 55-108.
- McClure, T. T. 1950. Anatomical aspects of the Fusarium wilt of sweet potatoes. *Phytopathology* **40**: 769-775.
- McLean, F. T. 1921. A study of the structure of the stomata of two species of *Citrus* in relation to Citrus canker. *Bull. Torrey Botan. Club* **48**: 101-106.
- Melander, L. W., and J. H. Craigie. 1927. Nature of resistance of *Berberis* spp. to *Puccinia graminis*. *Phytopathology* **17**: 95-114.
- Pierson, C. F., and J. C. Walker. 1954. Relation of *Cladosporium cucumerinum* to susceptible and resistant cucumber tissue. *Phytopathology* **44**: 459-465.
- Pool, V. M., and M. B. McKay. 1916. Relation of stomatal movement to infection by *Cercospora beticola*. *J. Agr. Research* **5**: 1011-1038.
- Powers, H. R., Jr. 1954. The mechanism of wilting in tobacco plants affected by black shank. *Phytopathology* **44**: 513-521.
- Rice, M. A. 1927. The haustoria of certain rusts and the reaction between host and pathogen. *Bull. Torrey Botan. Club* **54**: 63-153.
- Robertson, H. T. 1932. Maturation of foot and root tissue in wheat plants in relation to penetration by *Ophiobolus graminis* Sacc. *Sci. Agr.* **12**: 575-592.
- Sakurai, Y. 1952. Pathologico-anatomical observation on the white root rot of mulberry trees caused by *Rosellinia necatrix* (Hart.) Berl. *Research Repts. Fac. Textile Sericult. Shinshu Univ.* **2**: 18-26.
- Samuel, G. 1927. On the shot-hole disease caused by *Cladosporium carpophilum* and on the "shot-hole" effect. *Ann. Botany (London)* **41**: 375-404.
- Schaefer, R. 1932. Das Schicksal der Bakterien in den Knöllchen von *Lupinus albus* nebst cytologischen Untersuchungen. *Centr. Bakteriöl. Parasitenk. Abt. II* **85**: 416-425.
- Sharville, E. G. 1936. The nature of resistance of flax to *Melampsora lini*. *J. Agr. Research* **53**: 81-127.
- Shaw, L. 1934. Studies on resistance of apple and Rosaceous plants to fire blight. *J. Agr. Research* **49**: 283-313.

- Shimada, N. 1957. Relation of the development of mechanical tissues in rice leaves to the form of leaf smut spots. *Proc. Assoc. Plant Protect. Hokuriku Japan* **5**: 12.
- Smith, G. 1900. The haustoria of the Erysiphaceae. *Botan. Gaz.* **29**: 153-184.
- Stevens, F. L. 1922. The Helminthosporium foot-rot of Wheat, with observations on the morphology of Helminthosporium and the occurrence of saltation in the genus. *Illinois State Nat. Hist. Survey Bull.* **14**: 77-185.
- Struckmeyer, B. E., C. H. Beckman, J. E. Kuntz, and A. J. Riker. 1953. Plugging of vessels by tyloses and gums in wilting oaks. *Phytopathology* **44**(3): 148-153.
- Suzuki, H. 1951. Studies on the relation between the susceptibility of rice plant to blast disease caused by the low soil temperature and its anatomical and physiological characters. *Shokubutsu Byôgai Kenkyu Kyoto* **4**: 46-54.
- Suzuki, N. 1957. Studies on the violet root rot of sweet potatoes caused by *Helicobasidium mompa* Tanaka. VI. Histochemical studies of the infected tissues. (1) Chemical changes as results of infection. *Bull. Natl. Inst. Agr. Sci. (Japan) Ser. C* **8**: 69-130.
- Suzuki, N., Y. Doi, and S. Toyoda. 1953. Histochemical studies on the lesions of rice blast caused by *Piricularia oryzae* Cav. II. On the substance in the cell-membrane of rice reacting red in color with diazo reagent. *Ann. Phytopathol. Soc. Japan* **17**: 97-101.
- Suzuki, N., K. Kasai, T. Araki, and T. Takahashi. 1957. Studies on the violet root rot of sweet potatoes caused by *Helicobasidium mompa* Tanaka. 1. The disease invasion under field conditions. *Bull. Natl. Inst. Agr. Sci. (Japan) Ser. C* **8**: 1-27.
- Takahashi, Y. 1956. Studies on the mechanism of the resistance of rice-plants to *Piricularia oryzae*. II. Pathological changes microscopically observed in host cells in which fungus hyphae do not grow well. *Bull. Yamagata Univ. (Agr. Sci.)* **2**: 37-51.
- Tisdale, W. H. 1917. Flax wilt: a study of the nature and inheritance of wilt resistance. *J. Agr. Research* **33**: 845-872.
- Tochinai, Y. 1951. The functional resistance in plants. *Shokubutsu Byôgai Kenkyu Kyoto* **4**: 1-17.
- Tokunaga, Y., and M. Yokohama. 1955. Latent infections associated with some fruit diseases. Jubilee Publication in Commemoration of the Sixtieth Birthdays of Professors Tochinai and Fukushi. Hokkaido Univ., Sapporo. pp. 249-254.
- Tomiya, K. 1956. Cell physiological studies on the resistance of potato plant to *Phytophthora infestans*. IV. On the movement of cytoplasm of the host cell induced by the invasion of *Phytophthora infestans*. *Ann. Phytopathol. Soc. Japan* **21**: 54-62.
- Torigata, H. 1957. Studies on the mechanism of disease outbreak of black spot disease of Japanese pear. *Nagoya Univ. (Mimeographed)* pp. 1-117.
- Tullis, E. C. 1935. Histological studies of rice leaves infected with *Helminthosporium oryzae*. *J. Agr. Research* **50**: 81-90.
- Watanabe, T. 1939. On the anatomy of sweet potatoes affected by the stem rot fungus. *J. Agr. Research Soc. Utsunomiya Agr. Coll.* **14**: 65-75.
- Weimer, J. L., and L. L. Harter. 1921. Wound-cork formation in the sweet potato. *J. Agr. Research* **21**: 637-647.
- Woodward, R. C. 1927. Studies on *Podosphaera leucotricha* (Ell. et Ev.) Salm. I. The mode of perennation. *Brit. Mycol. Soc. Trans.* **12**: 173-204.
- Wylie, R. B. 1930. Cicatrization of foliage leaves. I. Wound responses of certain mesophytic leaves. *Botan. Gaz.* **90**: 260-278.

- Wylie, R. B. 1931. Cicatrization of foliage leaves. II. Wound responses of certain broad-leaved evergreens. *Botan. Gaz.* **92**: 279-295.
- Yoshii, Ha. 1936. Pathological studies on rice blast caused by *Piricularia oryzae*. II. On the mode of infection of the pathogen. *Ann. Phytopathol. Soc. Japan* **6**: 205-218.
- Yoshii, Ha. 1941a. Studies on the nature of rice blast resistance. I. The effect of silicic acid to the resistance. *Bul. Sci. Fak. Terkult., Kyushu Imp. Univ. Fukuoka, Japan* **9**: 277-291.
- Yoshii, Ha. 1941b. Studies on the nature of rice blast resistance. II. The effect of combined use of silicic acid and nitrogenous manure to the toughness of the leaf blade of rice and its resistance to rice blast. *Bul. Sci. Fak. Terkult., Kyushu Imp. Univ. Fukuoka, Japan* **9**: 292-296.
- Yoshii, Ha. 1944. Studies on black rot of sweet potatoes. II. Environmental conditions for the formation of wound cork. *Bult. Sci. Fak. Terkult. Kyushu Imp. Univ. Fukuoka, Japan* **11**: 129-138.
- Yoshii, Ha. 1948. Patho-histological observation of tomato inoculated with *Piricularia oryzae*. *Ann. Phytopathol. Soc. Japan* **13**: 14-18.
- Yoshii, Ha., and E. Kawamura. 1947. "Anatomical Plant Pathology." Asakura, Toyko.
- Yoshii, Hi. 1949. Comparative phytopathologic anatomical observation on the citrus leaves attacked by two types of the circular spotted leaf cast. Studies on the leaf cast of citrus trees (1). *Seibutsu* **4**: 112-115.
- Yoshii, Hi. 1957. Studies on the disease resistance in rice plants. *Mem. Ehime Univ. Sect. VI.* **3**: 1-149.
- Young, P. A. 1926. Facultative parasitism and host ranges of fungi. *Am. J. Botany* **13**: 502-520.

## CHAPTER 12

# Physiology and Biochemistry of Defense

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## I. INTRODUCTION

We cannot yet provide a wholly satisfactory account of the chemical basis of defense by higher plants against potential or invading pathogens. We do know, however, that a chemical basis for these phenomena exists. There is mounting evidence that the health of many plants is preserved not by virtue of mechanical barriers nor escape from infection but through an active metabolic initiative which destroys or immobilizes the pathogen at some stage before it can produce serious disease. There is even unassailable evidence that for many plants defense is not prepared in advance but depends upon metabolic events brought into play and substances



produced upon the approach of the pathogen to its prospective host. This chapter will be a guide to the nature and extent of evidence for the existence of biochemical mechanisms of defense rather than a compendium of established biochemical facts. It will attempt to evaluate the main hypotheses and the adequacy of present evidence concerning the origin and nature of these mechanisms. It will be less an account of substances and more an account of where to look for the substances or other agents of defense. Further information and ideas concerning the basis of plant defense are contained in several recent works (Gäumann *et al.*, 1950; Kern, 1956 a, b; Brown, 1955; Garrett, 1956; Walker and Stahmann, 1955).

The interaction of host and pathogen is one example of the pervasive struggle for existence which underlies much of the behavior of organisms and largely determines their present genetic potentialities. The processes involved in this interaction comprise a phase of ecology, differing from most ecological situations only in the intimate physical associations which are involved in the interplay of host and pathogen. As an aspect of the interactions among organisms, defense can only be conceived in terms of both participants in the drama, in terms of a host and a potential pathogen. Each phase of the struggle must be expressed in terms of an interaction and not in any absolute terms. Thus the outcome of an approach between two organisms may be expressed in terms of the susceptibility of one organism to the advance and harmful action of the other, or in terms of the virulence of one organism in its attack on another; but neither susceptibility nor virulence may be expressed as an attribute of one organism independently of others.

The reaction of a host to a particular pathogen is a property of the host capable of varying between two extremes, immunity at one extreme and complete susceptibility at the other. Between these two extremes is an indeterminate number of stages of increasing susceptibility as one progresses away from immunity, and of increasing resistance as one moves in the opposite direction along the scale, away from complete susceptibility. If successive segments of such a scale are cut off and given numbers from 0 (immune) to 4 or 5, a numerical designation of the degree of susceptibility is obtained. Such a scale has been widely used by plant pathologists for scoring reaction types. The terms "resistant" and "susceptible" and the scale showing degrees of susceptibility are designations of one organism's reaction to another.

The same individual plant will show the whole range of properties from immunity to susceptibility if its reaction to several different pathogens is considered successively. Efforts to arrange plants in categories of greater or lesser resistance have been unsuccessful for this reason, just as

any effort to arrange proteins in the order of their catalytic activity would be impossible if substrate were not considered in the assessment of activity. Although the potentiality for a reaction may exist independently of the pathogen, the actual chemical combinations and the chemical agents which give rise to visible results should not, *a priori*, be regarded as existing independently of a pathogen. The possibility must also be considered that the actual weapons of defense are a consequence of the interaction between host and pathogen. The very terminology which has been adopted in discussing plant disease is evidence that the consequences of the association of two organisms, and not preexisting substances in the host, are believed to be the determinants of parasitic attack. Thus the terms "defense," "resistance," and "reaction" all carry an implication of activity on the part of the host.

Considerations similar to those just presented for the host reaction must also apply to the pathogen, with appropriate care in distinguishing between the action of the pathogen in proliferating through the host, and its action in producing disease.

Nearly a century ago, Robert Koch adopted and made famous a few basic rules which served as a guide to the experimental measures needed to show that a specific organism was the causal agent of a disease. These rules, or as they have frequently been called, "postulates," required that: (1) a particular organism be found always in association with this disease; (2) this organism be isolated and obtained in pure culture outside the host; (3) on introducing this pure culture back into a healthy susceptible host the disease be produced; and (4) with the disease so produced, the organism should be constantly associated. Koch's rules can also be profitably applied to the problem of establishing the causal role of a chemical agent in producing the symptoms of disease (cf. Chester, 1933). They are in effect a statement of scientific procedure, and with appropriate adaptations they can be applied in general to the experimental proof of the causal nature of an agent, biological or chemical, suspected of producing an observable phenomenon or reaction. To adapt these rules to the problem of establishing that a certain chemical substance is responsible for protection against a disease it should be established that: (1) the substance is associated with the protection against this disease, at the site where protection occurs; (2) the substance can be isolated from hosts engaged in protection against the disease; (3) introduction of the substance to the appropriate loci of a healthy susceptible host confers protection; and (4) the nature of the protective action so induced resembles that of the natural agents of a resistant plant.

The application of these rules in the experimental attack on problems of plant protection may first require a broad definition of the agents of

protection. Thus it may be more appropriate, although experimentally more troublesome, to regard the agent of protection as a metabolic process, which would then require that experiments be done with separated enzyme systems. The productive application of these rules will be easier with some pathogenic associations than with others, but in all instances their successful application will require previous knowledge of the locus of defense reactions and the nature of the action against the pathogen—whether inhibition of spore germination, of stomatal penetration, of vegetative growth, or other actions. It will also require a hypothesis as to the nature of the chemical agent which might play a crucial role in defense. If this chapter can provide a picture of the clues now available to the origin and possible nature of these agents, it will have presented a fair view of the present status of this subject.

## II. THE CHEMICAL BATTERY

### A. *Preformed Antibiotics*

#### 1. *The Occurrence in Soil and Water of Toxic Substances Released from Higher Plants*

During the growth and accompanying activities of a higher plant there is a continuous exchange of materials with the surrounding environment, with a consequent modification of that environment. To other organisms nearby this fact may have profound significance, as in the supply of  $O_2$  which is returned by the green plant in exchange for the  $CO_2$  of respiration or in the amino acids which may be excreted from leguminous nodules and become available for other organisms in the rhizosphere of the legume (Virtanen and Laine, 1935). It is known that many other substances may be returned through the root to the soil water, such as nucleotides, flavones, hexose sugars, and inorganic ions (Lundegardh and Stenlid, 1944). Through the leaves both organic and inorganic substances are excreted, to be washed off onto the soil in periods of rain or heavy dews (Arens, 1929; Lausberg, 1935). These substances which become a part of the chemical environment act as stimulants or deterrents to the further development of the plant itself and contribute to the rise and fall of populations or individuals of other species (Lucas, 1949).

#### 2. *The Role of Diffusible Substances in Preventing or Retarding Infection*

There are a few well-documented instances of the participation of such substances in limiting the development or occurrence of higher plants. Transcinnamic acid from guayule plants, and 3-acetyl-6-methoxybenzaldehyde from the desert shrub *Encelia farinosa* are particularly

well-established agents of inhibitory action against higher plants (Bonner, 1950).

The suppression of pathogenic microorganisms by plant excretions also plays a role in protecting higher plants against competition from other organisms. As in the inhibition of growth of higher plants, substances excreted from root or shoot may have a more or less selective inhibitory action against microorganisms. Where such excretions occur, they may play an important role in the struggle for existence through their therapeutic action on the local environment. In general, protection which is conferred solely by such means is evident as a decrease in the number of loci of infection. If the chemical barrier is passed and an occasional locus of infection established, the local lesion or the colony which develops is just as large as on unprotected organs. This mechanism of protection should not, therefore, be expected when resistance is evidenced by a reduced development of the pathogen or its lesions occurring with similar frequency on resistant and on susceptible host varieties.

To establish experimentally that a plant which remains free of a potential pathogen owes its health to the production and release of a diffusible substance requires that these facts be established: (1) that diffusates, obtained from plants which are protected against the pathogen, are inhibitory to the phase of vegetative development of the pathogen which normally establishes the infection; (2) that the inhibitory agent from diffusates can be isolated under conditions precluding major production or loss during isolation; (3) that administration of the substance to the region which is the normal route of attack confers protection on an otherwise unprotected plant, and (4) that the action under these circumstances resembles the normal acts of protection. These facts are more amenable to experimental treatment for this type of resistance than for others.

The studies of Walker and co-workers on the onion smudge provide the most thoroughly documented accounts of the way in which chemical control by diffusible substances can operate (Walker and Stahmann, 1955). Varieties of onion with pigmented outer scales are usually resistant to smudge, caused by *Colletotrichum circinans*, while varieties with colorless scales are susceptible (Walker, 1923). Removal of the dry scales, however, abolishes resistance, and these varieties then become susceptible. Other variations in resistance are correlated with the presence of the intact colored dead scales. If spores of the pathogen are sown in infection drops on the colored scales, their germination is prevented by substances which have diffused out of the colored dead scale cells; but spores are not prevented from germinating on the scales of susceptible varieties. It is clear that the resistance depends upon a diffusible



toxic substance which if present wards off the fungus by inhibiting germination of the spores, by which infection is normally effected.

The inhibition of spore germination by extracts is accompanied by a second more definitive action, the bursting of spores or young hyphae with release of the protoplasmic contents (Walker, 1923). This property enabled Walker and his co-workers to follow the active substance by germination assays with an additional criterion to prevent the search from going astray. This might be hard to avoid if inhibition of germination were the sole criterion for the presence of the active substance.

The activity was obtained in water extracts of the colored scales but not of uncolored scales. After extraction, the colored scales were no longer toxic to spores germinating on them. These extracts were concentrated and from them crystals of a highly active component were obtained (Angell *et al.*, 1930) and identified as protocatechuic acid (Walker *et al.*, 1929; Link *et al.*, 1929a, b). This component did not account for all of the activity, but for a considerable part of it. Catechol was also identified in the extracts, and accounted for some of the inhibitory activity. Pure protocatechuic acid showed the same characteristic bursting of cells of the pathogen.

Although the inhibitory action of the dry scales is correlated with pigmentation, the pigments themselves (flavones and anthocyanins) are not inhibitory, nor are living cells which contain the pigments. Pigmented scales, therefore, become toxic only upon death, and the toxicity, although correlated with pigmentation, is not attributable to the pigments directly but to a colorless component released only upon death. Although toxic extracts can be obtained by crushing the scales, the activity obtained in this way bears no relation to the resistance of the fleshy scales to disease (Walker *et al.*, 1925). Nor does the toxic material of the dry scales provide a barrier to invasion by pathogens which penetrate by other routes, such as *Fusarium* root rot, the causal organism of which enters through the root scars. Resistance to other diseases caused by parasites penetrating via the scales is correlated with the presence of the colored scales only to the extent that the causal organism is sensitive to the soluble inhibitors. Thus, attack by *Aspergillus niger*, whose germination and growth are not inhibited by protocatechuic acid, is independent of scale color and the associated differences in phenol content, whereas resistance to *Diplodia natalensis*, penetrating via the dry scales, shows the same relation to color as does resistance to smudge (Ramsey *et al.*, 1946).

These experiments leave little doubt of the causal role of phenols, particularly protocatechuic acid, in resistance to onion smudge.

The study of the role of root exudates in relation to parasitism has been facilitated by two techniques whose use has led to some of the most instructive data on this subject. These techniques involve (1) some adaptation of the method of soil perfusion and (2) the use of collodion membranes to serve as artificial roots (Timonin, 1941). The former allows the collection of the excretions of the root while the plant is growing under natural conditions, supplied with nutrients and well aerated, and makes it relatively easy to compare excretions at different times. The latter provides an excellent technique for testing the activity of excretions under conditions approaching those of the natural plant root.

From observations on the microbial populations in the rhizosphere of resistant and susceptible flax varieties, Timonin (1940) concluded that excretions of the flax roots might be related to resistance to wilt caused by *Fusarium oxysporum* f. *lini*. He found larger populations of both bacteria and fungi in the rhizospheres than in the soil farther out from the roots, but the difference was greater for susceptible (var. Novelty) than for resistant plants (var. Bison). Flax plants were then grown in sterile culture solutions and the solutions with their accumulated excretions were tested for activity in several ways. They were placed in artificial roots, made of collodion membranes in the form of hollow cylinders, and these membranes were then immersed in moist soil. Stimulation of the microflora occurred as with the natural roots, more around the excretions from susceptible plants. Tests in agar cultures showed greater toxicity or less stimulation of *Fusarium oxysporum* and some other fungi by preparations from resistant plants. On the other hand, *Trichoderma*, which is itself antagonistic to *Fusarium*, flourished better in the diffusates from resistant flax than in those from the susceptible variety. The differential sensitivity of *Trichoderma* and *Fusarium* could, therefore, contribute to the net action of the resistant plants in keeping out a soil pathogen. Unfortunately Timonin did not examine the effect of these diffusates in suppressing inocula of the pathogen, or in protecting susceptible plants against infection. He did, however, go on to show that the diffusates from resistant varieties contained HCN in quantities (80 p.p.m.) which were demonstrated to be sufficient to inhibit growth in culture of *Fusarium oxysporum* f. *lini* but not *Trichoderma*. Susceptible varieties released no detectable cyanide. These results are of particular interest because of the fact that in another host-pathogen association, snow mold of barley, the damage to the host is produced by cyanide released by the pathogen (Lebeau and Dickson, 1953). The potential importance of *Trichoderma* spp. in suppressing the growth of

other soil fungi was demonstrated earlier by Weindling's isolation of an antibiotic (gliotoxin) produced particularly at low pH and capable of acting under soil conditions (1934, 1941).

Buxton (1957) has produced evidence that part of the defense of pea seedlings against another *Fusarium*, *F. oxysporum* f. *pisi*, the cause of pea yellows, also depends upon diffusible materials from the resistant varieties. He studied three varieties of pea showing genetic differences in resistance toward three races of the wilt fungus, and found a positive correlation between resistance to a given race and toxicity of root diffusates from healthy plants toward germination of the spores of the same race. The same diffusates which were strongly inhibitory toward an avirulent race were less inhibitory to a virulent race. The effects did not extend to other fungi tested, nor to the vegetative growth of the pathogen. The amount of inhibitory activity released in perfusion experiments was greatest at the time of abundant extrusion of lateral roots. Thus the diffusates of a healthy plant seem to contribute, in a way which is governed by genetic composition, to the specific action against physiologic races; but as Buxton points out, resistance to these pathogens is not wholly localized at the root surface.

Although the root may be an exceptionally leaky part of the plant, through the regions of metabolic transfer and through the holes pierced by the emerging lateral roots, other organs also excrete considerable quantities of solutes. These are known to include substances with marked effects on the germination and growth of some pathogens which normally gain entry through the aerial parts of the plant. Miss Lausberg's measurements of the water soluble cuticular excretions showed that extraordinarily large amounts of salts ( $\text{Ca}^{++}$  and  $\text{K}^+$ ) could pass through the plant and out onto the leaf surface, particularly when periods of rapid transpiration alternated with periods of heavy dew formation or rain (1935). The fine structure of the leaf surface, examined by electron microscopy of Formvar casts from the intact leaf have shown that in young leaves the deposits on the outer surface are continuously added to and renewed by extrusion, although this renewal declines with age (Mueller *et al.*, 1954; Schieferstein and Loomis, 1956). The leaves of many plants, if simply washed with water for brief periods, yield aqueous extracts which inhibit the germination of many fungus spores (Topps and Wain, 1957; Kovacs, 1955; Kovacs and Szeöke, 1956; Martin *et al.*, 1957).

In a study of the origin of differences in resistance of varieties of sugar beet to leaf spot, Kovacs found that low incidence of local lesions on the leaves of a resistant host variety was correlated with the presence of diffusible inhibitors from healthy leaves. Fewer spores of *Cercospora*

*beticola*, the causal fungus, germinate on the resistant leaves, and a correspondingly smaller percentage of these spores germinate in dew or water washings collected from best leaves. These water extracts also inhibit the growth of the germ tubes and they are active even after considerable dilution. Since resistance to *Cercospora* leaf spot consists mainly in permitting fewer spots to develop, it is possible that such external antibiotics may provide a major part of the defense against *Cercospora*, whose germ tubes must reach and enter a stoma as a prerequisite to the formation of an infection spot.

There are a number of plant diseases which are established primarily as a consequence of stomatal entry by the pathogen. Protection against such pathogens can be achieved by any action or inaction which prevents stomatal entry. For example, Isaac and Smith (1957) found that detached sunflower cotyledons establish few colonies of *Puccinia helianthi* when inoculated, although the attached cotyledon becomes heavily infected from a similar inoculation. Detachment of the cotyledon with a small bit of stem at the node, however, is sufficient to allow infection. This nodal tissue seems to play a part in determining whether the establishment of a rust colony will occur. The conditions which regulate stomatal entry appear to depend largely on the activities of the living guard cells, which will elicit appressorium formation and stomatal penetration even on isolated strips of epidermis if the guard cells are alive. For another pathogen, *Plasmopara viticola*, Arens showed that epidermal excretions played an important part in establishing infection, and that the positive chemotaxis on epidermal strips occurred only when the guard cells were alive (1929). The activity coming from the guard cells was attributed to surface active materials accumulating in the interphase between stomatal gas and infection drop. In view of the growing knowledge of these substances which regulate spore behavior, it should soon be possible to define more precisely some of these indirect mechanisms of defense, depending not so much on inhibitions as on the lack of a positive action.

Evidence has been presented by Martin *et al.* (1957) that inhibitory substances obtained from the surface of leaves can confer resistance when redeposited on otherwise susceptible leaves. They extracted the waxes from the leaf surfaces of apple varieties (Worcester-Permain and Cox's Orange Pippin) resistant to powdery mildew, *Podosphaera leucotricha*, and then deposited films of these waxes on the leaves in an aqueous solution together with a wetting agent. Conidia were then inoculated onto the leaves and examined for germination after 48 hours. The acidic fraction of the ether-soluble wax prevented germination of mildew conidia on apple leaves, and protected *Vicia faba* leaves against devel-



opment of disease lesions when inoculated with *Botrytis fabae*. The main component separated by chromatography was a phenol similar in chromatographic and color reactions to a fungitoxic substance which has appeared in water washings of the leaves of many trees and in the leaf and root excretions of *Vicia faba* (Topps and Wain, 1957). It would be interesting to know whether similar activity is lacking in the wax from susceptible stocks and whether the removal of leaf waxes from resistant leaves could be done so as to make those leaves temporarily susceptible and then apply resistance as was done with the inherently susceptible stock.

The foregoing discussion of excretions or diffusates playing a role in the deterrent action of the plant is not exhaustive, but includes some of the best documented studies that have been made so far. These examples suffice to show that a contribution to defense is made through the toxicity of these excretions, and that the toxicity is usually not highly specific. The action of these substances can be regarded as chemical exclusion, preventing the pathogen from reaching the portals of infection where the active struggle comes into existence. The action of several of these inhibitory materials has been tested on other fungi and they have been found active against nonpathogens as well as pathogens, suggesting that their action as inhibitors is not associated with specialized mechanisms of virulence, but with unspecialized properties of microorganisms. Only a little evidence is available that indicates any participation of preexisting diffusible metabolites in differentiating between genetically resistant and susceptible varieties of host plants.

### 3. Toxic Substances in the Cells of Resistant Plants

Once a pathogen reaches the tissues of a higher plant it may there encounter chemical conditions unfavorable for further development, even though the primary steps in infection have been successfully completed. The broad basis of immunity or resistance to many potential pathogens may well depend upon toxic materials which are preformed in the cells and tissues of plants. The kind of toxic material which could provide a basis for the preinfectious differentiation of resistant and susceptible plants must be a substance which occurs in the former, and is lacking or present in smaller amounts in the latter.

Substances toxic to microorganisms can be obtained from all kinds of plants. Hardly a microorganism exists whose development cannot be drastically hindered by suitable concentrations of an extract or of a substance derived from any one of numerous flowering plants. The economic and noneconomic flora and the laboratory shelves abound in toxic organic compounds, and even if they are scarce in an intact plant,

they are abundant in the breis that can be obtained by appropriate maceration and incubation of plant tissues. The finding of a substance toxic to a pathogenic fungus does not, therefore, necessarily signify that it is of importance in the resistance mechanisms of the plant from which it is derived.

Good evidence that an intracellular compound plays a part in the defense against disease is much harder to obtain than evidence for diffusible substances. It is not sufficient to establish that a resistant plant yields a substance toxic to the pathogen toward which it shows resistance, nor even to show a correlation between resistance and yield of toxic material. Such evidence is suggestive, but if the substance inhibits virulent and avirulent strains alike, or if the substance is not found at the portal of infection, or in the concentrations required to inhibit, it can hardly provide a convincing explanation for the differentiation between resistant and susceptible plants. The greater difficulties of the experimental approach are partly responsible for the paucity of well-documented evidence for a role of preformed cellular inhibitors in defense. The evidence that phenols are related to rust resistance (Newton *et al.*, 1929; Newton and Anderson, 1929) has never led to a clear-cut demonstration of the part which these compounds actually play. Similarly, the discovery of a fungitoxic alkaloid, tomatin, in tomato plants led to the suggestion that its presence was related to disease resistance (Irving *et al.*, 1945; Irving, 1947). When concentrations occurring in resistant plants were determined, however, and compared with the concentrations required for inhibition, there appeared to be insufficient alkaloid to account for resistance (Kern, 1952). Virtanen and his co-workers have published a long series of papers on the oxazolinones of plants and their fungicidal activity (Virtanen *et al.*, 1957), and although the variety of derivatives and the large amounts of these substances present in some plants make the possibility of their protective function intriguing, there is as yet little evidence that they are the substances responsible for resistance to microorganisms producing disease. Virtanen's school has also characterized a number of other fungistatic compounds found in plants, but the inhibitory concentrations are high. The knowledge of the distribution and changes during development which their work has provided for compounds such as chlorogenic, gallic, and benzoic acid and their derivatives may be most useful in determining how much of a role these substances play in resistance, but their work has not yet provided convincing evidence of such a role.

If the distribution of a toxic compound is shown to be correlated with resistance, the probability that it plays a role in defense is much greater. Such a correlation exists between the distribution of chlorogenic acid in

potato tubers and resistance to scab (*Streptomyces scabies*). Higher concentrations of chlorogenic acid occur in resistant than in susceptible varieties; the compound is largely confined to the outermost tissues where the scab organism normally proliferates; and the tissues around the lenticels, where infection occurs, are higher in chlorogenic acid than other parts of the peel (Johnson and Schaal, 1952). The action of chlorogenic acid is related by these investigators to its stimulation of cork cambium activity and the rapid establishment of a protective layer of cork.

The implication in resistance of phenols and their derivatives, particularly chlorogenic acid, has been repeatedly proposed, and there are numerous records of the occurrence of phenols in resistant plants and in infected tissues. Recently, there has been a tendency to ascribe the importance of these compounds not to their occurrence and toxic action directly, but to their appearance and conversion into toxic substances in response to infection (Valle, 1957). This aspect of the part played by phenols will be discussed further in connection with dynamic aspects of defense.

#### *B. Protection Which Depends on the Lack of an Essential Substance*

An unfavorable chemical environment in a potential host may consist of a deficiency of an essential substance rather than a toxic level of an inhibitor. For a specific pathogen, the substances which it encounters must include all essential nutrients and any substances whose formative effects are instrumental in successful infection. The potential importance of nutrient deficiencies within the host in the limitation of parasitic development has been elaborated by Lewis (1953) and by Garber (1956) and some striking instances have been reported by Garber (1954); Garber and Goldman (1956), and Keitt and co-workers (Keitt and Boone, 1956; Boone *et al.*, 1957; Kline *et al.*, 1957). Even when the essential nutrients are present, a parasite may fail to develop if the nutrients are not available, and maceration of the tissues may then create a more favorable chemical environment (Garber and Goldman, 1956). In a series of classic experiments dealing with this question, Keitt and his co-workers have produced mutants of *Venturia inaequalis*, the apple scab fungus, each of which has been demonstrated to have a genetically conditioned requirement for a growth factor. Several of these mutants differ from the wild type in the loss of pathogenicity, and when inoculated onto the leaf in the usual manner they fail to establish colonies. In some of these, pathogenicity can be restored by administering the required growth factor to the infection court. This is an elegant demonstration of the thesis that pathogenicity depends on an adequate supply by the host of all nutrients required by the parasite, and that a sort of

passive resistance may be provided by a deficiency of one such nutrient. It should be pointed out, however, that not all of the nutritional mutants would become pathogenic when the required nutrient was supplied, so that even in this parasite-host complex other factors besides nutrients play a determining role in disease development.

Another type of protection based on the absence of materials required for infection is exemplified by the seedling diseases caused by *Pellicularia filamentosa* (*Rhizoctonia filamentosa*). Infection requires the formation of a hyphal cushion at the surface of the host epidermis, and entry is then accomplished by penetration at the site of such a mycelial cushion. When susceptible seedlings (lettuce, radish) are grown aseptically in cellophane bags, *Pellicularia* forms mycelial cushions on the outside of the cellophane, but when resistant seedlings (tomato) are grown in the bags, no cushions are formed (Kerr, 1956). Sterile excretions from the susceptible plants also induce cushion formation in cultures of the corresponding pathogenic strains of *Pellicularia* growing on cellophane disks, but not in cultures of nonpathogenic strains (Kerr and Flentje, 1957). These cushions do not allow penetration of the cellophane, but in the presence of suitable exudate do allow penetration of epidermal strips of the congenial host, so that there appears to be some other property of the epidermis which is needed for successful establishment of the pathogen. There is in these experiments good indication that the absence of specific formative substances provides a passive protection against this fungus. Conversely, susceptibility may be regarded as a more positive attribute depending upon the production of certain formative substances by the host, or upon the production of substances by the host preventing a defense reaction from coming into play. The chemical nature of these substances is not yet known.

The hyphal cushions which here play a part in establishing the infection are characteristic of the response of *Rhizoctonia* to many higher plants. They occur in the cells of orchids infected with symbiotic *Rhizoctonia*, and Noel Bernard believed that "the key to the problem of immunity must be in the factors which determine hyphal cushion formation" (1909, p. 156). This view is of particular interest since the hyphal cushions to which it refers are in orchids evidence of successful defense, whereas in the seedling diseases they appear to be a major instrument in the successful breaching of defenses.

Another example of the same type of protection, depending upon the absence of substances required in the coordination of the infection process, is provided by diseases which are initiated by stomatal entry. As discussed earlier, the coordination of these events is usually brought about by the healthy plant, but its breakdown under any circumstances confers resistance.



### *C. Genetic Factors Determining Resistance*

Soon after the rediscovery of Mendel's laws, at the beginning of the 20th century, several biologists interested in problems of disease recognized the importance of Mendelian factors in conditioning the reaction to pathogens. Although it was realized previously that resistance was passed from parent to offspring, it became possible with a knowledge of dominance, linkage, and independent assortment of genetic factors to demonstrate convincingly a close genetic control of resistance in some plants. Biffen (1907, 1912) and Orton (1908) were leaders in this work, which was carried forward vigorously and capably by a number of men in the United States, Europe, and Russia. It was soon established that immunity from infection in many plants for many pathogens was inherited with simple monohybrid or dihybrid ratios, and there developed rapidly the extensive agronomic application of these findings to the development of crop plants resistant to most of the major pathogens (see Vavilow, 1953, for a comprehensive account of the work up to the 1930's). In a general discussion of the implications of these findings in 1911, Freeman already realized that the heritable factors were in many cases not reflected in visible properties or structures of the resistant plant, but instead acted as determinants of the reaction to a pathogen. "Protoplasmic factors," not structural or mechanical factors, have thus long been recognized as the intermediaries of the genes in bringing about the expression of resistance. Resistance to obligate parasites (rusts, powdery mildews, downy mildews, etc.) and to facultative parasites (smuts and bunts, anthracnose, potato blight, wilts, and other fusarial diseases, as well as a majority of other diseases investigated) has turned out to be genetically determined, often with a single genetic locus conditioning resistance or susceptibility. How direct is the chemical connection between the gene and the arrest of development of the nonvirulent pathogen? At least it cannot be the chromosomal determinant itself which is involved in the reaction with the approaching parasite, since the arrest of the pathogen usually occurs without its contacting the host nucleus. Actually, there is abundant evidence that the host does not at first behave as though it were going to react against the invader. The early development of many kinds of pathogens proceeds on the resistant and susceptible hosts at a similar pace and with a similar apparently congenial reception by the host (Scheffer and Walker, 1954; see Allen, 1954, for references to obligate parasites). It is, therefore, unlikely for many of these parasites that the actual toxic substances are preformed in the host. Instead, the trigger mechanism represented by the host gene or its products is the agent which is preformed, this trigger being re-

leased with the production of toxic materials only after contact with the parasite. Recent developments in nuclear physiology suggest that either nucleic acid components or proteins might be early products of gene action, and that the specific genetic differences between resistant and susceptible plants might, therefore, be reflected in differences in such components. In the case of animal blood antigens, each genetic difference has a corresponding protein antigen difference (Irwin, 1953), and there is considerable evidence for other organisms also that specific enzymes are formed or not in accordance with the presence or absence of the corresponding gene (Wagner and Mitchell, 1955). These facts provide grounds for speculating that specific proteins might be preformed in the host to correspond to each gene concerned with resistance and susceptibility. Recent preliminary attempts to distinguish differences in the proteins of resistant and susceptible plants gave evidence of more positively charged proteins in the susceptible than in the resistant plants (wheat reaction to leaf rust: Barrett and McLaughlin, 1954). The amino acids of pairs of wheat embryos isogenic except for a resistance factor possessed by one of the pair showed no significant differences, however (wheat embryos selected for reaction to smut: Zscheile and Murray, 1957).

Although the highly specific genetic control that has been found means that some highly specific chemical events are involved in starting the defense reactions, this does not mean that the actual agents of protection must be specific. The products of interaction between host and pathogen may well include some nonspecific substances, elicited by a variety of different confrontations of host and pathogen, and acting in a common way. The actual arsenal of defense need not be as varied as the conditions which cause its development.

### III. DYNAMIC ASPECTS OF DEFENSE

#### *A. Induced Production of Diffusible or Small Molecular Inhibitors*

##### *1. Pathogen-Induced Diffusible Inhibitors*

To understand the nature of the dynamic defense which comes into play in response to attempted invasion, the host must be studied after it has begun to interact with the pathogen. Comparative chronological studies of host plants beginning before the interaction and continuing through the period when the defensive response emerges can then help in the recognition of the changes and substances whose appearance signals the success of a defense reaction. By such studies carried out under a variety of conditions, correlations can be established which provide the physiological basis for the formulation of a biochemical hypothesis.

The associations most amenable to experimental analysis are, therefore, those which show the interaction before actual physical contact or which can be brought into physical contact and then subsequently separated.

One of the earliest studies of the nature of defense reactions was initiated by Noel Bernard, whose studies of the mycorrhizae of orchids are noteworthy for their careful execution and for their insight. Caullery (1952) refers to them as "magnificent researches." Bernard was impressed by the retarded growth of mycorrhizae following the initial rapid spread into an embryo and by the protection which one infection provided against a subsequent infection (1909). These phenomena he interpreted as "acquired immunity," and he regarded the failure of endophytic mycorrhizae to penetrate beyond the roots into the tubers as a further evidence of immunity in the latter organ. To investigate the nature of this immunity, he studied the interaction of mycorrhizal fungi with aseptically excised tuber pieces, planted—at some distance apart—on gelatin media (1911). As an example of his results, he found that with tissue from *Loroglossum hircinum* and the fungus *Rhizoctonia repens* (isolated from *Orchis morio*), the mycelium started to grow in all directions but was soon sharply arrested in its advance on the side toward the tuber piece. Since this fungistatic activity was prevented by heating the tuber at 55° C. for one-half hour, Bernard believed the active material to be heat labile. Grinding the tuber prevented the activity from appearing, and no activity was observed unless the tuber piece was larger than 0.5 cm.<sup>3</sup> From Bernard's experiments, which because of his early death were not brought to a conclusion, it is not clear whether the active material arises from the cut tissues in response to something from the fungus, or whether it is already present before confrontation with the fungus in culture. Magrou (1924) repeated and continued these experiments. He planted the tuber pieces on gelatin and left them there for some time, then removed the pieces and inoculated the gelatin with *Rhizoctonia repens*. Again, the fungus failed to grow into the area where the tuber had been, and Magrou concluded, therefore, that the active substance was preformed in the tuber. Nobécourt (1929), studying *Loroglossum* and the endophyte which he succeeded in isolating from it (*Rhizoctonia hircini*, called by him *Orcheomyces hircini*), found little activity of the killed tuber pieces alone, but marked activity from tuber pieces which were exposed to the fungus, then killed and tested for diffusible toxic substances. More recently, Gäumann and co-workers (1950) have carried out a careful reinvestigation of this important question, and have confirmed Nobécourt's conclusions. Working with *Orchis militaris* and its endophyte, *Rhizoctonia repens*, they have found that some toxicity occurs in the tuber pieces without laboratory exposure to

the fungus, and that confrontation with the fungus in culture leads to an enhanced production of toxic substances, which could be demonstrated in the agar after removal of the "induced" tuber piece. These experiments leave little room for doubt that substances diffusing from the endophyte cultures lead to an enhanced production by the tuber of fungistatic substances. The possibility that the wounding involved in all of these experiments is solely responsible for the increased toxicity is eliminated, but it is still possible that the wounding contributes to the high levels of toxicity. It would be interesting to test tubers from uninfected plants, if such could be obtained by feeding with concentrated sugar solutions, but the experiments already done constitute one of the most convincing bodies of evidence now available for the induction of defense by diffusible substances produced in a higher plant in response to the oncoming microorganism. There is as yet no information available concerning the chemical nature of either the diffusible inducing substance(s) or of the toxic material produced, but the experiments show that the latter continues to be released from the host tissue for some time after its induction. Although these experiments concern a symbiotic association, their broader significance was clearly recognized in Bernard's statement that "La symbiose est à la frontière de la maladie."

A second series of investigations which have provided important evidence for the production of defense substances only after interaction of fungus and higher plant has been carried out by K. O. Mueller and his co-workers. Mueller did much of the pioneer work on the genetics of resistance of the Irish potato to late blight caused by *Phytophthora infestans*. He soon became interested in the physiological basis of the genetic differences and looked for the cause of the difference in response between resistant and susceptible hosts. The course of the reaction, although somewhat similar in the two kinds of hosts, differs particularly in the speed of response (1939). In resistant plants inoculated with zoospores of *Phytophthora*, a rapid response occurs and host cells die before the fungus has a chance to become established, while the slower response of susceptible plants allows time for growth and reproduction. When the hypersensitive fleck is formed in resistant plants, the fungus is killed. The same thing happens when zoospores are inoculated onto other flowering plants which do not become diseased with *Phytophthora*: necrotic flecks are produced and the fungus is prevented from proliferating (1950). This phenomenon is also of widespread occurrence elsewhere among the pathogens of higher plants. From observations on the course of microscopic changes, Mueller postulated the formation of fungitoxic substances, "Phytoalexinen," (1939; Mueller and Borger, 1940), by loose analogy with the alexins of animal blood which are in-



volved in combination with antigens. Preliminary experiments with potato tissue and *Phytophthora* zoospores indicated that the contact between potato and spores of an avirulent strain of blight fungus led to the production of substances also toxic to a virulent strain as well as to other fungi. Thus, the toxic materials once produced did not seem to be specific for any particular microorganism (Mueller and Behr, 1949). In contrast to the pathogen specificity required if preexisting agents of defense determine resistance, agents of defense brought into existence only upon infection need not show specificity.

An elegant method for studying these substances under aseptic conditions and without interference from wound substances was devised by Mueller (1956). Drops of a suspension of *Phytophthora* zoospores were placed on the aseptically exposed sterile inner epidermis of young bean pods, and after incubating them aseptically, the drops were collected and tested for the appearance of soluble substances causing bursting or affecting germination or germ-tube growth of test spores. Control drops without spores were handled and tested in the same way. These experiments showed that drops incubated in contact with bean and fungus gave rise to antibiotic substances which were lacking in the drops without fungus spores. The possibility that the active substances came from the spores was not systematically excluded, as it could have been by incubating another series of drops with spores on an inert substratum. In further tests with another fungus, *Sclerotinia fructicola*, it was found that toxic materials did not appear until after about one-half day of incubation, and that they failed to appear on bean pods previously warmed to 41° C. This latter experiment argues against the active materials coming from the fungus itself.

These experiments are particularly interesting because they have been carried out without contamination by extraneous organisms and without interference from extraneous substances formed when tissues are mechanically wounded. They represent the best approach yet made to the question of the occurrence and nature of substances produced in the defense reaction and responsible for arresting the progress of a pathogen. The active materials involved are fungistatic in low and fungicidal in high concentrations; they are dialyzable and stable to freezing and boiling, but their chemical properties have not yet been investigated. If the approach can be applied to combinations of pathogen and its natural host, it should provide a potent tool for studying the chemical basis of the hypersensitive reaction and of the protection against pathogenic attack which it provides (cf. Chapter 13).

A number of other investigations have produced evidence of the formation or enhancement of fungistatic substances in response to

invasion. Increases occur in the toxicity of potato upon inoculation with *Helminthosporium carbonum* (Kuč *et al.*, 1955, 1956). The increased toxicity cannot be accounted for on the basis of chlorogenic and caffeic acid contents in the infected tissue (Kuč, 1957). Toxicity to *Fusarium oxysporum* appears in extracts of pea seedlings after inoculation with *F. solani* f. *pisi*, while neither extracts of healthy seedlings nor culture filtrates of the pathogen alone are toxic (Buxton, 1957). Increased inhibition of spore germination of *F. bulbigenum* by the juices from rhizomes of tubers after infection with this fungus has also been reported (Shimomura *et al.*, 1955). The uninvaded tissues of sweet potato infected with *Ceratostomella fimbriata* produce ipomeamarone, chlorogenic acid, and other phenols, and some of these compounds are highly toxic to the fungus in culture (cf. Chapter 10). All of these findings suggest that the production of toxic substances occurs even when resistance is lacking or is incomplete. Most investigations have included a report on the changes in phenolic compounds, and there is no doubt that phenols may be present in higher levels in those tissues which have reacted with the pathogen, but this is not invariably so (e.g., polyphenols decrease in the lotus rhizome, and may also decrease in potato tuber infected with *Phytophthora infestans* (Tomiya *et al.*, 1958). The conclusion has been reached with increasing accord that it is not the actual level of phenol which is the most important factor, but the metabolic changes in which phenols are involved.

## 2. Injury as the Ultimate Cause of Defense

Whenever a plant sustains injury locally, a series of defense reactions come into play which tend to repair the damage (Went, 1940; Bloch, 1952, 1953). The nature of the response is characteristic of the tissue affected, and the same response may occur with a variety of different wounding agents. There is a good deal of evidence that substances released from injured cells are involved in triggering the defense reactions to mechanical injury. These wound substances, or as they have been called "hormones," initiate processes leading to new cell divisions and sometimes to the formation of new types of tissue, particularly cork. In the evolution of a mechanism of defense against pathogenic organisms, it is probable that the general potentialities for coping with injury have been applied to the special job of coping with parasitic injury. It is, therefore, to be expected that the defense against pathogens will have some of the same features as the defense against nonspecific injury. The first of these features which the two processes share is that the agents of defense are fashioned only after the cause of the injury has acted. Since some of the procedures employed in studying defense reactions

involve wounding of the tissue, special care must be taken to establish that the pathogen, and not a mechanical wound, is the ultimate cause of production of defense materials. Some authors have realized that the toxic phenols obtained from tissues in the process of reacting to a foreign organism might have arisen from the wound reaction (Spencer *et al.*, 1957). There is also experimental evidence that the natural defenses against a pathogen may be enhanced by wounding the host (Keyworth and Dimond, 1952).

All of these findings lead to the conclusion that similar phenolic compounds may be produced by a variety of injurious agents, mechanical or biological. They are frequently produced by the experimental manipulations which are used to study them. Perhaps they are released from conjugated forms present within the cells of most plants, and by virtue of their fungistatic action constitute a general protective agent. The peculiar property of allowing free development of a pathogen would then be the special attribute of the plant susceptible to that pathogen, and would require some provision for avoiding the release or accumulation of toxic materials.

### B. *Induced Immunity by Antibody Formation*

In the last years of the 19th century and the beginning of the 20th century, work in animal immunology gave a powerful impetus to the search for the possible occurrence and basis of plant immunity. Unfortunately, the search for the basis of plant immunity was pushed forward in advance of evidence for its occurrence, with the result that a great body of literature appeared dealing with the question of antibody formation in plants and predicated upon procedures adopted for work with animals. Much of this work is unsound, and a great deal of the presumed evidence for the occurrence of antigen-antibody reactions is spurious, arising from nonspecific precipitations and agglutinations of plant extracts. The philosophical and procedural basis of this work was thoroughly analyzed, experiments were conducted to test the validity of some crucial points, and the results were summarized in an important survey by K. S. Chester in 1933. This work placed the problem in a more realistic perspective. The reaction to his able and exhaustive review of the field seems to have been to discourage any further exploration, for the subject has not since received any appreciable attention. As Chester pointed out, however, the possibility of a phenomenon in plants analogous to acquired immunity in animals is not ruled out. The examples discussed above constitute specific instances of the occurrence of such a phenomenon based upon diffusible substances. The possibility that immunity based upon induction of specifically reacting proteins also occurs in

plants is more difficult to establish, but it also cannot be ruled out. There seems no good reason to believe that such immunity, if it exists, will appear in the same way or be amenable to the same experimental procedures that it is in animals. In fact, it is rather more likely that procedures adapted to the many structural and functional differences between higher plants and higher animals would have to be developed. This can only be done satisfactorily by taking as a starting point plant-pathogen associations in which it is demonstrated that acquired immunity occurs, and by proceeding with these to find what the chemical basis of the acquired immunity may be. Whether the investigation leads to evidence for an antigen-antibody reaction of the type that occurs in warm- and some cold-blooded animals or not is immaterial. The important outcome would be the discovery of the actual basis of acquired plant immunity. The pitfalls that beset the earlier exploration of this field could be largely avoided now, and yet the motivations for exploring its possibilities are, if anything, greater than ever because of recent confirmation of the high degree of specificity which lies behind the violent interaction between uncongenial hosts and avirulent pathogens. One of the most remarkable features of this specificity is that it depends not only on specific genes of the host, but also on correspondingly specific genes in the pathogen (Flor, 1956). In this respect it is analogous to the compatibility reactions between pollen tube and stigma tissues (Lewis, 1954).

In the field of protection against virus infection, the classic work of Salaman (1933, 1938) provided well-documented evidence for the induction of protection to subsequent infections by earlier inoculations of virus. The mechanism of such induced protection against viruses will undoubtedly be explored further.

### *C. Relation of Metabolic Changes to the Processes of Defense*

#### *1. Occurrence of Changes in Main Phases of Metabolism Following Infection*

The alterations in kinds and levels of substances which occur in response to infection are a consequence of changes in host metabolism. Marked changes in the major metabolic processes take place in infected plants, and include changes in protein metabolism and particularly striking changes in the respiratory metabolism (Allen, 1953 and 1954; Rubin and Arzichowskaja, 1953; Rubin *et al.*, 1955; Farkas and Király, 1958; Chapter 10 of this volume). These changes have been studied most thoroughly in susceptible plants which exhibit the characteristic symptoms of disease and only recently has a concerted attempt been initiated to make a comparative study of respiratory changes in resistant and sus-



ceptible plants after inoculation. Evidence to date indicates no difference between resistant and susceptible plants in the initial response, but generally the rise in the rate of  $O_2$  uptake in resistant plants reverses earlier than in susceptible ones (Samborski and Shaw, 1956). Any attempt to correlate respiratory changes with resistance must, however, involve more than a comparison of over-all rates of metabolism, since quantitative comparisons of rates of respiration may fail entirely to reveal the real differences. The idea that a shift in the relative rates of different aspects of metabolism might be triggered by the pathogen and result in the development of an unfavorable chemical environment has been elaborated particularly by Sempio (1950).

## 2. *Relation of Altered Metabolism to the Emergence of Defense*

a. *Metabolic Detoxification.* One of the early proposals concerning the role of metabolism suggested that the host protected itself against disease by enzymatic detoxification of the harmful metabolites of a pathogen. This was A. N. Bach's idea concerning the protective role of oxidative enzymes (Rubin and Arzichowskaja, 1953; Farkas and Király, 1958). The idea was based on the fact that pronounced increases in oxidations occur when tissues are injured and on the appearance of high oxidase activity in the injured zones. Some evidence for metabolic detoxification has been obtained with animal pathogens (cf. Agner, 1950), and more recently this phenomenon has been implicated in the protection against the toxin of *Helminthosporium victoriae*. Resistant oats infiltrated with the toxin, victorin, are not affected, and the toxin cannot be recovered from resistant tissues (Romanko, 1958). Since the proposal of metabolic detoxification would only account for freedom of the host from deleterious effects of the pathogen and not for freedom from proliferation of the pathogen, it appears, however, to be inadequate to account for most aspects of resistance. It may have more bearing on the problem of toleration of symbionts than upon the successful defense against parasitic invasion.

b. *Metabolic Formation of Toxins.* The data presented in the earlier sections of this chapter provide strong evidence that the protective action of the host may arise from the formation of substances toxic to the pathogen rather than on the removal of substances deleterious to the host. The "peaceful co-existence," as it is aptly called by Farkas and Kiraly, of the first stages of infection is terminated by an arrest in the development of the pathogen. It seems, therefore, essential that an explanation of resistance be sought in an antagonistic action against the pathogen itself, not simply against its metabolites. This mode of action was suggested by Cook *et al.* (1911), who proposed that the toxic sub-

stances were oxidation products of such compounds as tannic acid. If expanded to include host metabolism in general, the suggestion provides a good working hypothesis on which several lines of investigation have been based. The idea envisages some triggering action of the pathogen upon the host, presumed to be chemical and leading to the formation of an agent which alters the course of metabolism. The altered course of metabolism is the immediate source of fungistatic or other antagonistic action. This alteration may even be triggered by an avirulent pathogen and the resulting host reaction then provides protection against a virulent strain (Mueller, 1953).

The importance of the host tissue in bringing the toxic substances into existence is indicated by a quantitative relation between the amount of host tissue involved in the reaction and the achievement of defense. In the case of orchid tuber pieces discussed earlier, a minimal amount of tissue is required to develop the fungistatic substances. If the piece is smaller, it is overgrown by the mycorrhizal fungus which a larger piece would stop (Bernard, 1911; Gäumann *et al.*, 1950). Mueller's observations on the potato blight organism discussed earlier indicated that a difference in rate of reaction determined degree of resistance, with a rapid reaction leading to quick arrest of the pathogen and high resistance (avirulent strains). More direct evidence for the importance of the amount of substance produced per unit time comes from the work of Tomiyama *et al.* (1958). The action of a certain amount of inoculum in giving rise to resistance was found to be a function of the thickness of potato slice, increasing resistance occurring with increasing thickness up to about 2 mm. under the conditions of their experiments. They calculated that it took about ten host cell layers to achieve full resistance in tuber slices of a genetically resistant variety. Since the locus of resistant action was at the surface in all cases, the differences appear to depend on products contributed by underlying cells. No relation was found between increase in polyphenol and resistance, but more rapid removal of polyphenol and greater accumulation of brown products of oxidation were observed in the tissues displaying higher resistance.

Tomiyama's studies show, in agreement with many others, that the mass effect of increased inoculum is in the opposite direction, acting to overcome resistance. Thus with larger inocula thin slices become quite susceptible, whereas with smaller inocula they retain some of their resistance. This effect is not to be confused with the effect of heavy inoculum acting directly on the pathogen to prevent its development (Yarwood, 1956).

c. *Other Sources of the Unfavorable Action of Altered Metabolism.*  
In the absence of definitive evidence concerning the biochemical relation

between altered metabolism and resistance, the possibility must be left open that neither of the above chemical models (paragraphs a and b) will prove completely satisfactory and that some other formulation of the relationship will prove necessary. It may be preferable, therefore, to regard the metabolic changes as the source of defense without a commitment as to how this end result is achieved. This permits a continued analysis of the aspects of metabolism which are involved in defense and of the causal relationship between metabolism and defense, with the question of the mechanics of the relationship to be determined by later experimental findings.

### 3. *The Nature of the Metabolic Changes Leading to Production of the Defense Conditions*

One of the most persistent views concerning the metabolic origin of defense is the view that it arises from an alteration in oxidase activity. The experiments of Cook showing that gallic acid became toxic when mixed with plant juices containing oxidizing enzymes were among the earliest which urged this view. This was later fortified, and attention directed to the importance of polyphenoloxidase in particular, by the studies of Szent-Györgyi and Vietorisz (1931), who showed that phenoloxidase activity greatly increased upon injury to tissues. The toxicity of the quinones formed was suggested by them as a basis for the protective action of this enzyme. In the presence of suitable reducing systems (such as ascorbate or the cellular dehydrogenases) these quinones would not accumulate. The focus of attention on quinones seemed to be justified because this class of compounds is known to include many toxic substances (McNew and Burchfield, 1951). Schaal and Johnson (1955) demonstrated a correlation between the autoxidation occurring at higher pH values and the toxicity of a group of phenols. Those phenols which were autoxidizable, as indicated by the appearance of color, were toxic, but only at pH values where autoxidation occurred. There is, therefore, experimental evidence that oxidation of phenolic compounds can produce substances of greater toxicity than the original phenol.

The defense reactions of the plant, both to wounding and to pathogens producing a hypersensitive reaction, may lead to the formation of colored products; and the formation of such products is associated with the activation of polyphenoloxidase. An increase in polyphenoloxidase activity is a common aspect of pathogenic infection. An increase in the relative activity of polyphenoloxidase (Rubin *et al.*, 1955) or in the absolute activity (Menon and Schachinger, 1957), has been reported to be greater in resistant than in susceptible plants. These circumstances have provided a body of evidence supporting the idea that an augmented activity

of polyphenoloxidase occurring during infection is causally related to the production of toxic substances and that these protect against the pathogen which initiates their formation. These substances are presumed to accumulate when the increased rate of oxidation by  $O_2$  is not associated with a similar increase in the activity of dehydrogenase systems effecting the reduction of quinones to phenols.

There are, however, some apparently contradictory facts and some weaknesses in this concept which must be considered seriously. One is the fact that phenol oxidases are generally inactive in intact tissue and the amount of activity in extracts is, therefore, not an indication of the activity in the tissue. There is not convincing evidence that this kind of oxidase is involved in activating oxygen in respiration (Bonner, 1957). A second objection is the failure of phenoloxidase action in many instances to produce toxic substances. In tests of a large series of phenols, Rich and Horsfall (1954) found that phenoloxidase action upon these compounds resulted in decreased toxicity rather than increased toxicity which is to be expected from the proposed role of phenoloxidases in resistance. The failure to develop toxicity could be due to a rapid conversion of quinones to polymerization products (colored products formed after oxidation of the phenol to a quinone). These polymerization products have, by some investigators, been regarded as including the toxic materials. There is, however, no good reason to suppose that it is the final brown and red products of polymerization which are toxic, since these could just as well be the products of secondary reactions associated with a different defensive reaction. Colored products are not always formed when resistance is exhibited (Hirai, 1956), and even in the case of Mueller's phytoalexins, the toxic solutions have little color and are themselves causes of the fleck reaction in the host as well as of the fungistatic action toward the pathogen, although the two activities are not known to reside in the same compound. It is not possible at present to attribute the protective action of the reacting host to any particular products of phenoloxidase action, even though some of these products may be toxic. Some of the studies with respiratory poisons also throw further doubt on the uniqueness of the role of phenoloxidase.

Important evidence concerning the nature of the metabolic processes involved in resistance has been obtained recently by studies with respiratory poisons. It has been found that a resistant plant may be made susceptible by certain inhibitors. Gassner and Hassebrauk found increased susceptibility to rust in wheat treated with chloroform (1938). Resistance to some potato pathogens is broken down by narcotics, and this action is associated with inhibition of the ability to form wound cork (Behr, 1949). Further studies of this phenomenon have shown that the



defense of potato varieties against blight can be modified by infiltration with a number of different metabolic inhibitors or substrates (Fuchs and Kotte, 1954; Christiansen-Weniger (geb. Kotte), 1955). Inhibitors of metal oxidases generally reduce the resistance, as do several organic acids and, remarkably, also catechol and tyrosine. Although most of the substances which inhibit polyphenoloxidase also prevent resistance, the action against this enzyme does not seem to be essential in the breakdown of resistance. In another host-pathogen association, *Fusarium* wilt of tomato, for which similar information is available, resistance is broken down by thiourea, diethyldithiocarbamate, sodium fluoride, ethanol, and 2, 4-dinitrophenol (Gothoskar *et al.*, 1955). Streptomycin, 2, 4-D, maleic hydrazide, and other physiologically active compounds are known to interfere with resistance and allow the development of a pathogen even at concentrations which inhibit the pathogen in culture. The number of such observations is now sufficiently numerous to leave no doubt that the development of the resistant reaction is dependent on metabolic events which can be so altered as to abolish completely the normal protective response of the host plant, but they have not yet provided clear evidence of the metabolic process on which this response depends. Further investigations along these lines should prove fruitful in elucidating the metabolic basis of resistance.

Another phase of these studies on metabolism and resistance which may deserve attention is the possible role of the direct oxidative pathway in creating the defensive opportunities for the infected plant. Evidence is rapidly accumulating that the enhanced respiration of infected tissues results from the opening of this metabolic pathway in a variety of diseases (Farkas and Király, 1955; Daly *et al.*, 1957; Daly and Sayre, 1957; Shaw and Samborski, 1957). The dehydrogenases involved in this metabolic route are linked to triphosphopyridine nucleotide (TPN) whereas those involved in oxidations via pyruvate and the Krebs cycle are predominantly linked to diphosphopyridine nucleotide (DPN). The latter are known to couple with molecular oxygen by way of the cytochrome oxidase system, but whether the  $\text{TPNH}^*$  generated in the course of the direct oxidative pathway is oxidized by molecular oxygen with the help of cytochrome oxidase, or ascorbic oxidase, or some other oxidase is not yet clear, but there are indications that it may go by way of a copper oxidase (Király and Farkas, 1957). In monocotyledons the copper oxidase found is generally ascorbic acid oxidase, whereas either ascorbic acid oxidase or phenoloxidase may occur in dicotyledons.

There are several reports which present evidence that implicates the growth hormones in the process of induced development of resistance.

\* Reduced triphosphopyridine nucleotide.

The decreased resistance to rust caused by irradiation of the shoot apex (Schwinghamer, 1957), indications of altered resistance to wilt in tomatoes caused by ionizing radiation (Waggoner and Dimond, 1956) and by 2, 4-dichlorophenoxy acetic acid, naphthaleneacetic acid, and several other growth regulators (Davis and Dimond, 1953), and increased resistance to rust caused by administration of indoleacetic acid (Samborski and Shaw, 1957), all point to the participation of growth hormones in the emergence of effective defense reactions. The general trend of these results suggests that higher auxin levels are associated with defense, but this is not easily reconcilable with the fact that decreased indoleacetic oxidase and hence increased auxin levels are found in susceptible plants (Shaw and Hawkins, 1958; Pilet, 1957).

#### IV. CONCLUSION

The data now available concerning the plant's defense against pathogenic attack point to one main conclusion regarding the nature of defensive action: it is not a condition of the plant which constitutes resistance, but a process of response. The potentialities for response are inherent, but the successful employment of these potentials depends first of all on the specific nature of the triggering agent, but more importantly on a series of metabolic events which are set in motion by the pathogen. Whether these events lead to successful defense or to some other result depends on the context and may be experimentally altered so as to reverse the outcome completely. It is probable that some of the chemical events are of great importance in the creation of an unfavorable chemical environment and that certain kinds of chemical agents, transient or otherwise, are recurring products of the metabolic events set in motion by the pathogen or by other agents and are instrumental in arresting the development of a potential pathogen. Neither the toxic substances nor the metabolic processes which lead to their formation are the specific agents corresponding to the specific pathogen and host genes involved in determining the interaction. This specificity must be sought in the initial triggering interaction.

The problem of compatibility between a pathogen and its host is one phase of a very broad biological problem. Phenomena of compatibility and incompatibility come into play in many biological processes (Lewis, 1954). In the fusion of gametes; in the growth of the pollen tube in the style; in the fusion of protoplasm of slime molds and of cells of fungus hyphae, both in anastomosis and in sexual reproduction; in graft acceptance or rejection; and in the important realm of reactions in animals to foreign substances, as well as in many other aspects of the coordinated living and acting together of cells, compatibility phenomena occur and have been shown to have a genetic basis. It is, therefore, distinctly pos-

sible that ideas about the basis of compatibility in pathogenic associations may be obtained from these other areas of biological investigation. Likewise, some of the advances in our understanding of incompatibility reactions (defense) in plants may contribute to a more integrated understanding of the universal basis which the common genetic foundation suggests that various compatibility phenomena may have.

## REFERENCES

- Allen, P. J. 1953. Toxins and tissue respiration. *Phytopathology* **43**: 221-229.
- Allen, P. J. 1954. Physiological aspects of fungus diseases of plants. *Ann. Rev. Plant Physiol.* **5**: 225-248.
- Agner, K. 1950. Studies on the peroxidative detoxification of purified diphtheria toxin. *J. Exptl. Med.* **92**: 337-347.
- Angell, H. R., J. C. Walker, and K. P. Link, 1930. The relation of protocatechuic acid to disease resistance in the onion. *Phytopathology* **20**: 431-438.
- Arens, K. 1929. Physiologische Untersuchungen an *Plasmopara viticola*, unter besonderer Berücksichtigung der Infektionsbedingungen. *Jahrb. wiss. Botan.* **70**: 93-157.
- Barrett, R. E., and J. H. McLaughlin. 1954. Disease resistance factors in wheat. *J. Agr. Food Chem.* **2**: 1026.
- Behr, L. 1949. Über den Einfluss von nekrotisch wirkenden Stoffen auf die Wundperidermbildung und Resistenz der Kartoffelknolle gegenüber *Phytophthora infestans* de By. und Vertretern der Gattung *Fusarium* Lk. *Phytopathol. Z.* **15**: 407-446.
- Bernard, N. 1909. L'évolution dans la symbiose. *Ann. sci. nat. Botan.* [9] **9**: 1-196.
- Bernard, N. 1911. Sur la fonction fungicide des bulbes d'Ophrydées. *Ann. sci. nat. Botan.* [9] **14**: 221-234.
- Biffen, R. H. 1907. Studies in the inheritance of disease resistance. I. *J. Agr. Sci.* **2**: 109-128.
- Biffen, R. H. 1912. Studies in the inheritance of disease resistance. II. *J. Agr. Sci.* **4**: 421-429.
- Bloch, R. 1952. Wound healing in higher plants. *Botan. Rev.* **18**: 655-679.
- Bloch, R. 1953. Defense reactions of plants to the presence of toxins. *Phytopathology* **43**: 351-354.
- Bonner, J. 1950. The role of toxic substances in the interactions of higher plants. *Botan. Rev.* **16**: 51-65.
- Bonner, W. D., Jr. 1957. Soluble oxidases and their functions. *Ann. Rev. Plant Physiol.* **8**: 427-452.
- Boone, D. M., D. M. Kline, and G. W. Keitt, 1957. *Venturia inaequalis* (Cke.) Wint. XII. Pathogenicity of induced biochemical mutants. *Am. J. Botany* **44**: 791-796.
- Brown, W. 1955. On the physiology of parasitism in plants. *Ann. Appl. Biol.* **43**: 325-341.
- Buxton, E. W. 1957. Some effects of pea root exudates on physiologic races of *Fusarium oxysporum* Fr. f. pisi (Linf.) Snyder and Hansen. *Brit. Mycol. Soc. Trans.* **40**: 145-154.
- Caullery, M. 1952. "Parasitism and Symbiosis." Sidgwick and Jackson, London. 340 pp.
- Chester, K. S. 1933. The problem of acquired physiological immunity in plants. *Quart. Rev. Biol.* **8**: 129-154; 275-324.

- Christiansen-Weniger (geb. Kotte), Eva. 1955. Versuche zur stoffwechselphysiologischen Beeinflussung der Reaction der Kartoffelknolle auf *Phytophthora infestans* de By. *Phytopathol. Z.* **25**: 153-180.
- Cook, M. T., H. F. Bassett, F. Thompson, and J. J. Taubenhaus. 1911. Protective enzymes. *Science* **33**: 624-629.
- Daly, J. M., and R. M. Sayre. 1957. Relations between growth and respiratory metabolism in safflower infected by *Puccinia carthami*. *Phytopathology* **47**: 163-168.
- Daly, J. M., R. M. Sayre, and J. H. Pazur. 1957. The hexose monophosphate shunt as the major respiratory pathway during sporulation of rust of safflower. *Plant Physiol.* **32**: 44-48.
- Davis, D., and A. E. Dimond. 1953. Inducing disease resistance with plant growth-regulators. *Phytopathology* **43**: 137-140.
- Farkas, G. L., and Z. Király. 1955. Studies on the respiration of wheat infected with stem rust and powdery mildew. *Physiol. Plantarum* **8**: 877-887.
- Farkas, G. L., and Z. Király. 1958. Enzymological aspects of plant diseases. I. Oxidative enzymes. *Phytopathol. Z.* **31**: 251-272.
- Flor, H. H. 1956. The complementary genic systems in flax and flax rust. *Advances in Genet.* **8**: 29-54.
- Freeman, E. M. 1911. Resistance and immunity in plant diseases. *Phytopathology* **1**: 109-115.
- Fuchs, W. H. and E. Kotte. 1954. Zur Kenntnis der Resistenz von *Solanum tuberosum* gegen *Phytophthora infestans* de By. *Naturwissenschaften* **41**: 169-170.
- Garber, E. D. 1954. The role of nutrition in the host-parasite relationship. *Proc. Natl. Acad. Sci. U. S.* **40**: 1112-1116.
- Garber, E. D. 1956. A nutrition-inhibition hypothesis of pathogenicity. *Am. Naturalist* **90**: 183-194.
- Garber, E. D., and M. Goldman. 1956. The response of grape tissue cultures to inoculation with biochemical mutants of *Erwinia aroideae*. *Botan. Gaz.* **118**: 128-130.
- Garrett, S. D. 1956. "Biology of Root-Infecting Fungi." Cambridge Univ. Press, London and New York. 293 pp.
- Gassner, G., and K. Hassebrauk. 1938. Untersuchungen über den Einfluss von Äther-und Chloroformnarkose auf das Rostverhalten junger Getreidepflanzen. *Phytopathol. Z.* **11**: 47-97.
- Gäumann, E., R. Braun, and G. Bazzigher. 1950. Über induzierte Abwehrreaktionen bei Orchideen. *Phytopathol. Z.* **17**: 36-62.
- Gothoskar, S. S., R. P. Scheffer, M. A. Stahmann, and J. C. Walker. 1955. Further studies on the nature of *Fusarium* resistance in tomato. *Phytopathology* **45**: 303-307.
- Hirai, T. 1956. Studies on the nature of disease resistance on plants. *Shokubutsu Byōgai Kenkyu* **5**: 139-157.
- Irving, G. W., Jr. 1947. The significance of tomatin in plant and animal diseases. *J. Wash. Acad. Sci.* **37**: 293-296.
- Irving, G. W., Jr., T. D. Fontaine, and S. P. Doolittle. 1945. Lycopersicin, a fungistatic agent from the tomato plant. *Science* **102**: 9-11.
- Irwin, M. R. 1953. Genes and antigens. In "Information Theory in Biology" (H. Quastler, ed.), Section 2b. Univ. of Illinois Press, Urbana, Illinois. pp. 147-169.
- Isaac, P., and J. Smith. 1958. Personal communication.
- Johnson, G. and L. A. Schaal. 1952. Relation of chlorogenic acid to scab resistance in potatoes. *Science* **115**: 627-629.



- Keitt, G. W., and D. M. Boone. 1956. Use of induced mutations in the study of host-parasite relationships. *Brookhaven Symposia in Biol.* **9**: 209-223.
- Kern, H. 1952. Über die Beziehungen zwischen Alkaloidgehalt und Krankheitsresistenz bei verschiedenen Tomatensorten. *Verhandl. schweiz. Naturforsch. Ges. (Basel)*: p. 150.
- Kern, H. 1956a. Problems of incubation in plant disease. *Ann. Rev. Microbiol.* **10**: 351-368.
- Kern, H. 1956b. Resistenz gegen Infektion. Immunität. In "Encyclopedia of Plant Physiology" (W. Ruhland, ed.), Vol. 2. Springer, Berlin. pp. 826-839.
- Kerr, A. 1956. Some interactions between plant roots and pathogenic soil fungi. *Australian J. Biol. Sci.* **9**: 45-52.
- Kerr, A., and N. T. Flentje. 1957. Host infection in *Pellicularia filamentosa* controlled by chemical stimuli. *Nature* **179**: 204-205.
- Keyworth, W. G., and A. E. Dimond. 1952. Root injury as a factor in the assessment of chemotherapeutants. *Phytopathology* **62**: 311-315.
- Király, Z., and G. L. Farkas. 1957. On the role of ascorbic oxidase in the parasitically increased respiration of wheat. *Arch. Biochem. Biophys.* **66**: 474-485.
- Kirkham, D. S. 1954. Significance of the ratio between the water soluble aromatic and nitrogen constituents of apple and pear in the host-parasite relationship of *Venturia* species. *Nature* **173**: 690-691.
- Kline, D. M., D. M. Boone, and G. W. Keitt, 1957. *Venturia inaequalis* (Cke) Wint. XIV. Nutritional control of pathogenicity of certain induced biochemical mutants. *Am. J. Botany* **44**: 797-803.
- Kovacs, A. 1955. Über die Ursachen der unterschiedlichen Resistenz der Zuckerrübensorten gegen *Cercospora beticola* Sacc. *Phytopathol. Z.* **24**: 283-298.
- Kovacs, A., and E. Szeöke. 1956. Die phytopathologische Bedeutung der kutikulären Exkretion. *Phytopathol. Z.* **27**: 335-349.
- Kuéc, J. 1957. A biochemical study of the resistance of potato tuber to attack by various fungi. *Phytopathology* **47**: 676-680.
- Kuéc, J., A. J. Ullstrup, and F. W. Quackenbush. 1955. Production of fungistatic substances by plant tissue after inoculation. *Science* **122**: 1186-1187.
- Kuéc, J., R. E. Henze, A. J. Ullstrup, and F. W. Quackenbush. 1956. Chlorogenic and caffeic acids as fungistatic agents produced by potatoes in response to inoculation with *Helminthosporium carbonum*. *J. Am. Chem. Soc.* **78**: 3123-3125.
- Lausberg, Th. 1935. Quantitative Untersuchungen über die kutikuläre Exkretion des Laubblattes. *Jahrb. wiss. Botan.* **81**: 768-806.
- Lebeau, J. B., and J. G. Dickson. 1953. Preliminary report on production of hydrogen cyanide by a snow-mold pathogen. *Phytopathology* **43**: 581-582.
- Lewis, D. 1954. Comparative incompatibility in angiosperms and fungi. *Advances in Genet.* **4**: 235-285.
- Lewis, R. W. 1953. An outline of the balance hypothesis of parasitism. *Am. Naturalist* **87**: 273-281.
- Link, K. P., H. R. Angell, and J. C. Walker. 1929a. The isolation of protocatechuic acid from pigmented onion scales and its significance in relation to disease resistance in onions. *J. Biol. Chem.* **81**: 369-375.
- Link, K. P., A. D. Dickson, and J. C. Walker. 1929b. Further observations on the occurrence of protocatechuic acid in pigmented onion scales and its relation to disease resistance in the onion. *J. Biol. Chem.* **84**: 719-725.
- Lucas, C. E. 1949. External metabolites and ecological adaptation. *Symposia Soc. Exptl. Biol.* **111**: 336-356.

- Lundegårdh, H., and G. Stenlid. 1944. On the exudation of nucleotides and flavanones from living roots. *Arkiv Botan.* **31**(10): 1-27.
- Magrou, J. 1924. À propos du pouvoir fungicide des tubercules d'Ophrydées. *Ann. sci. nat. Botan.* [10] **6**: 265-270.
- Martin, J. T., R. F. Batt, and R. T. Burchill. 1957. Defense mechanism of plants against fungi. *Nature* **180**: 796-797.
- McNew, G. L., and H. P. Burchfield. 1951. Fungitoxicity and biological activity of quinones. *Contribs. Boyce Thompson Inst.* **16**: 357-374.
- Menon, R., and L. Schachinger. 1957. Die Rolle des Phenols bei der Widerstandsfähigkeit von Tomatenpflanzen gegen Infektion. *Ber. deut. botan. Ges.* **70**: 11-20.
- Mueller, K. O. 1939. Physiologisch-genetische Untersuchungen über die Resistenz der Kartoffel gegenüber *Phytophthora infestans*. *Naturwissenschaften* **27**: 765-768.
- Mueller, K. O. 1950. Affinity and reactivity of Angiosperms to *Phytophthora infestans*. *Nature* **166**: 392-394.
- Mueller, K. O. 1953. The nature of resistance of the potato plant to blight-*Phytophthora infestans*. *J. Natl. Inst. Agr. Botan.* **6**: 346-360.
- Mueller, K. O. 1956. Einige einfache Versuche zum Nachweis von Phytoalexinen. *Phytopathol. Z.* **27**: 237-254.
- Mueller, K. O., and L. Behr. 1949. Mechanism of *Phytophthora*-resistance of potatoes. *Nature* **163**: 498-499.
- Mueller, K. O., and H. Borger. 1940. Experimentelle Untersuchungen über die *Phytophthora*-Resistenz der Kartoffel. *Arb. biol. Reichsanstalt Land-u. Forstwirtschaft. Berlin-Dahlem* **23**: 189-231.
- Mueller, L. E., P. H. Carr, and W. E. Loomis. 1954. The submicroscopic structure of plant surfaces. *Am. J. Botany* **41**: 593-600.
- Newton, R., and J. A. Anderson. 1929. Studies on the nature of rust resistance in wheat. IV. *Can. J. Research* **1**: 86-99.
- Newton, R., J. V. Lehmann, and A. E. Clarke. 1929. Studies on the nature of rust resistance in wheat. I-III. *Can. J. Research* **1**: 5-35.
- Nobécourt, P. 1929. "Contribution à l'étude de l'immunité chez les végétaux," J. Barker et Cie, Tunis. 176 pp.
- Orton, W. A. 1908. The theory and practice of breeding disease-resistant plants. *Am. Breeders Assoc. Ann. Rept.* **4**: 145-156.
- Pilet, P. E. 1957. Activité anti-auxines-oxydasique de *L'Uromyces pisi* (Pers.) de By. parasite d'*Euphorbia cyparissias* L. *Phytopathol. Z.* **31**: 162-179.
- Ramsey, G. B., B. C. Heiberg, and J. S. Wiant. 1946. *Diplodia* rot of onions. *Phytopathology* **36**: 245-251.
- Rich, S., and J. G. Horsfall. 1954. Relation of polyphenol oxidases to fungitoxicity. *Proc. Natl. Acad. Sci. U. S.* **40**: 139-145.
- Romanko, R. R. 1958. A physiological basis for resistance of oats to Victoria blight. (Unpublished manuscript.)
- Rubin, B. A., and E. W. Arzichowskaja. 1953. "Biochemische Charakteristik der Widerstandsfähigkeit der Pflanzen gegenüber Mikroorganismen." Akademie Verlag, Berlin. 87 pp.
- Rubin, B. A., E. P. Chetverikova, and E. V. Arzichowskaja. 1955. The oxidation system and the immunity of plants. (In Russian.) *Zhur. Obschei Biol.* **16**: 106-118.
- Salaman, R. N. 1933. Protective inoculation against a plant virus. *Nature* **131**: 468.

- Salaman, R. N. 1938. The potato virus "X": its strains and reactions. *Phil. Trans. Roy. Soc. London* **B229**: 137-217.
- Samborski, D. J., and M. Shaw. 1956. The physiology of host-parasite relations. II. *Can. J. Botany* **34**: 601-619.
- Samborski, D. J., and M. Shaw. 1957. The physiology of host-parasite relations. IV. *Can. J. Botany* **35**: 449-455.
- Schaal, L. A., and G. Johnson. 1955. The inhibitory effect of phenolic compounds on the growth of *Streptomyces scabies* as related to the mechanism of scab resistance. *Phytopathology* **45**: 626-628.
- Scheffer, R. P., and J. C. Walker. 1954. Distribution and nature of *Fusarium* resistance in the tomato plant. *Phytopathology* **44**: 94-101.
- Schieferstein, R. H., and W. E. Loomis. 1956. Wax deposits on leaf surfaces. *Plant Physiol.* **31**: 240-247.
- Schwinghamer, E. A. 1957. Effect of ionizing radiation on rust reaction in plants. *Science* **125**: 23-24.
- Sempio, C. 1950. Metabolic resistance to plant disease. *Phytopathology* **40**: 1-23.
- Shaw, M., and A. R. Hawkins. 1958. The physiology of host-parasite relations. V. *Can. J. Botany* **36**: 1-16.
- Shaw, M., and D. J. Samborski. 1957. The physiology of host-parasite relations. III. *Can. J. Botany* **35**: 389-407.
- Shimomura, T., A. Yamaguchi, I. Uritani, and T. Hirai. 1955. Resistance of lotus to the rhizome rot caused by *Fusarium bulbigenum* Wt. var. *nelumbicolum* N. et W. *Ann. Phytopathol. Soc. Japan* **20**: 47-53.
- Spencer, D. M., J. H. Topps and R. L. Wain. 1957. An antifungal substance from the tissues of *Vicia faba*. *Nature* **179**: 651-653.
- Szent-Györgyi, A., and K. Victorisz. 1931. Bemerkungen über die Funktion und Bedeutung der Polyphenoloxidase der Kartoffel. *Biochem. Z.* **233**: 236-239.
- Timonin, M. I. 1940. The interactions of higher plants and soil microorganisms. II. Study of the microbial population of the rhizosphere in relation to resistance of plants to soil-borne diseases. *Can. J. Research* **C18**: 444-456.
- Timonin, M. I. 1941. The interactions of higher plants and soil microorganisms. III. Effect of by-products of plant growth on activity of fungi and actinomycetes. *Soil Sci.* **52**: 395-413.
- Tomiyama, K., M. Takakuwa, and N. Takase. 1958. The metabolic activity in healthy tissue neighboring the infected cells in relation to resistance to *Phytophthora infestans* (Mont.) de By. in potatoes. *Phytopathol. Z.* **31**: 237-250.
- Topps, J. H., and R. L. Wain. 1957. Fungistatic properties of leaf exudates. *Nature* **179**: 652-653.
- Valle, E. 1957. On antifungal factors in potato leaves. *Acta. Chem. Scand.* **11**: 395-397.
- Vavilov, N. I. 1953. Study of immunity of plants from infectious diseases. In "The Origin, Variation, Immunity and Breeding of Cultivated Plants" (Trans. by K. S. Chester), Vol. 13, No. 1/6. *Chronica Botanica*, Waltham, Massachusetts. pp. 96-168.
- Virtanen, A. I., and S. Laine. 1935. Chemical nature of the amino acids excreted by leguminous root nodules. *Nature* **136**: 756-757.
- Virtanen, A. I., P. K. Hietala, and O. Wahlroos. 1957. Antimicrobial substances in cereals and fodder plants. *Arch. Biochem. Biophys.* **69**: 486-500.
- Waggoner, P. E., and A. E. Dimond. 1956. Altering disease resistance with ionizing radiation. *Phytopathology* **46**: 125-127.

- Wagner, R. P., and H. K. Mitchell. 1955. "Genetics and Metabolism." Wiley, New York. 444 pp.
- Walker, J. C. 1923. Disease resistance to onion smudge. *J. Agr. Research* **24**: 1019-1040.
- Walker, J. C., and M. A. Stahmann. 1955. Chemical nature of disease resistance in plants. *Ann. Rev. Plant Physiol.* **6**: 351-366.
- Walker, J. C., C. C. Lindegren, and F. M. Bachmann. 1925. Further studies on the toxicity of the juice extracted from succulent onion scales. *J. Agr. Research* **30**: 175-187.
- Walker, J. C., K. P. Link, and H. R. Angell. 1929. Chemical aspects of disease resistance in the onion. *Proc. Natl. Acad. Sci. U. S.* **15**: 845-850.
- Weindling, R. 1934. Studies on the lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathology* **24**: 1153-1179.
- Weindling, R. 1941. Experimental considerations of the mold toxins of *Gliocladium* and *Trichoderma*. *Phytopathology* **31**: 991-1003.
- Went, F. W. 1940. Local reactions in plants. *Am. Naturalist* **74**: 107-116.
- Yarwood, C. E. 1956. Cross protection with two rust fungi. *Phytopathology* **46**: 540-544.
- Zscheile, F. P., and Hazel C. Murray. 1957. Chromatographic study of amino acid development in wheat ovules in relation to genes for disease resistance. *Phytopathology* **47**: 631-632.





## CHAPTER 13

# Hypersensitivity \*

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\* In this chapter the hypersensitive reaction is abbreviated as HR and host-pathogen combination is abbreviated as H/P.

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## I. INTRODUCTION

The term hypersensitivity is borrowed from human medical terminology. Literally, it means that an organism or group of organisms is sensitive to a pathogenic agent beyond the norm. As can be seen from the following amplifications, the definition describes only one aspect of the phenomenon here under discussion. Moreover, the hypersensitivity concept in plant pathology differs essentially from the same term in human medicine. Thus, no analogous conclusions can be drawn from examples in animal pathology.

What does the phytopathologist understand by the term hypersensitivity and its synonyms (suprasensitivity, hypersusceptibility, hyperergy, Überempfindlichkeit, and in connection with these concepts: incompatibility reaction, protoplasmic resistance, active resistance, etc.)? It is suitable to start with the phenomenon itself, so that one does not become a slave to a preconceived terminology.

The Cambridge botanist, Ward, was the first to recognize clearly the significance of the hypersensitivity reaction as a defense mechanism of the plant against potential parasites. In his treatise (1902a) "On the relations between host and parasite in the bromes and their brown rust" he shows that the pathogen penetrates with its hyphae into resistant as well as susceptible hosts. He observed no differences between the behavior of the resistant and susceptible hosts until direct, physiologic contact is established. In the congenial host, "the parasite slowly taxes its host and even stimulates the cells for some greater activity." In the uncongenial host, the pathogen induces changes that were described by Ward as follows: "The tissues turned brown and died, the destructive action of the infecting tubes having killed the cells too rapidly." At the same time, the pathogen ceases to grow. Thereby, the infection is limited to a local necrosis and the plant escapes the disease. The writer attributes the premature physiologic breakdown of cells to an increased sensitivity of the tissue of the hosts toward some metabolic products of the parasite.

Ward recognized that both extremes "highest sensitivity" and "highest tolerance" of the host cell are connected by intermediary steps and

furthermore, that the mode of reaction is influenced by environmental factors. He tried in vain to answer the problem, not as yet solved, why a pathogen such as *Puccinia dispersa* discontinues its growth prematurely in the hypersensitivity host. He endeavored to find "some body" in the cell sap of the host plant which inhibits or promotes the growth of the fungus. These efforts failed. "Of course," he emphasizes, "the experiments teach very little." Finally, he suggested that "internal, i.e., intraprotoplasmic properties beyond the reach of the microscope and similar in their nature to those which bring about the essential differences between species and varieties themselves" must be the cause for the gradually changing behavior of the host plant.

About 10 years later, research on the phenomenon described by Ward received further impetus from Stakman. His discovery that Eriksson's "formae speciales" of the cereal rust fungus consist of many physiologic races, the host spectra of which are determined by the interaction mechanism described by Ward, gave direction to further progress in hypersensitivity research. The term, hypersensitivity, was introduced by Stakman into phytopathological terminology.

Since the hypersensitivity phenomenon was first studied on cereal rusts, the opinion prevailed until the 1920's that it is connected with plant diseases elicited by obligate biotrophic fungi. The causal interpretation of the mechanism of the process was also strongly influenced by this opinion. However, it was slowly recognized that facultative parasites, such as *Phytophthora infestans* or *Venturia inaequalis* can bring forth reactions in their hosts that have all the earmarks of the phenomenon described by Ward for the rust diseases. Virus research provided a further impetus, since it was found that viruses may multiply within a short time even in the uncongenial host, but they induce necrosis in the infected tissues, causing a localization of the infection.

Finally, and most recently, the hypersensitivity or hypersusceptibility concept has been applied to interactions of some sucking insects and their hosts, that lead to a premature necrosis of the sucked part of tissue and that prevent the establishment of a normal parasitic relationship.

In looking back on the history of hypersensitivity research three ways can easily be recognized by which attempts were made to gain insight into the nature of this phenomenon. There are numerous works at hand in which scientists tried to find by histological or tissue-physiological methods how the course of interaction differs in "hypersensitivity" from the "normosensitive" hosts (structural analysis). The second way consisted in examining how far the interaction is determined by the genetic



constitution of the host or pathogen and by environmental conditions (conditional analysis). And finally, attempts were made to find through physiological experiments and chemical analysis *the* factor that prevents the pathogen from developing in the tissue of the hypersensitive host after initial normal development (causal analysis).

Such separation can of course be maintained in theory. Actually, the approaches constantly overlapped, especially when histological findings were used to interpret the phenomenon.

In order to avoid misunderstandings, some definitions are expressed as follows:

1. Hypersensitivity reaction (HR) encompasses all morphological and histological changes that, when produced by an infectious agent, elicit the premature dying off (necrosis) of the infected tissue as well as inactivation and localization of the infectious agent.

2. A plant which does not react in a hypersensitive fashion and, therefore, is susceptible to the pathogen, is called "normosensitive." Depending on the intensity of necrotic changes with which the cell of the host responds to the infection, distinct degrees of sensitivity are distinguishable.

3. Interactions that lead to a rapid death of the cell of the host and simultaneously to inactivation of the pathogen are called "parabiotic" according to Gäumann (1948). On the other hand, in case of a parasitic relationship, in which the cells of the host and the pathogen remain alive for a longer period of time (mutual tolerance) the interaction is called "eusymbiotic."

4. The expressions "resistance" and "susceptibility" are used exclusively in a clinical sense. Thus, "resistance" only means that a certain plant—withstanding the presence of a particular disease producing agent and conditions favorable for infection—remains free or nearly free of the disease. The term "tolerance" is applied to hosts (e.g., latent virus carriers) that do not become ill despite clinical infections.

5. The terms "virulence" and "avirulence" are used, respectively, with "susceptibility" and "resistance." They always refer only to the underlying host/pathogen combination (H/P combination).

It is impossible to define exactly the term "hypersensitivity" itself, for we know by experience only that in the resistant host the interaction occurs with greater force than in the susceptible one. The conclusion that the resistant host must be "more sensitive" than the susceptible one to some material influences of the causative agent is no more than a supposition still in need of experimental study. As long as we do not know anything about the nature of the factor (or factors) that produces HR, each definition can be only of descriptive value.

## II. HYPERSENSITIVITY REACTION AS MORPHOLOGICAL PHENOMENON

Hypersensitivity interactions produced by fungal pathogens will be discussed first, because we are best informed about them, and because the results achieved through them have decisively influenced the course of research.

### A. Hypersensitivity Reactions Induced by Fungi

#### 1. Obligate Biotrophic Fungi \*

Apart from Archimycetes, that develop entirely within the cell of the host, intercellular or extramatrix hyphal growth is characteristic for obligate biotrophic fungi. The cytoplasm of the cell of the host comes almost exclusively into direct contact with the haustoria of the pathogen. Since it is the eusymbiotic combination where the pathogen and host apparently live together "peacefully," a mutual tolerance between the border areas of both partners should be postulated.

*Puccinia* type: According to Stakman and Levine (1922), at least 6 interaction types can be differentiated in rust diseases of cereals for which thorough information is available:

i = no macroscopically recognizable symptoms

0 = chlorotic or necrotic specks surrounded by a chlorotic rim area, no fructification of the causative agent

1, 2, and 3 = chlorotic and more or less necrotic specks with gradually diverse fructification of the causative agent

4 = no necrotic changes in the area of infected tissue, chlorosis only weakly indicated, optimal fructification of the causative agent.

A further interaction type, "x," was added to these six. It is characterized by the fact that three or more reaction types are realized on the same leaf (mesothetic type).

Extensive literature is available about changes that occur during interaction (e.g., Ward, 1902a; Marryat, 1907; Stakman, 1914; R. F. Allen, 1923, 1927; Noll, 1951). The following results are considered essential in this connection: The pathogen can penetrate into the plant, independently from interaction type. In infection type "i," interaction ends

\* The following definitions apply to this chapter: Biotrophic fungi are defined as those able to grow exclusively in living tissue (e.g., rust fungi). Fungi thriving in living tissue without killing it but able to grow on dead substrata are referred to as facultative biotrophs (e.g., smut fungi). The term necrotrophic is reserved for pathogens that spread in host tissues, killing it if the infection is not halted by the hypersensitive reaction (e.g., *Colletotrichum lindemuthianum*).

in a necrosis with only microscopically recognizable cell complexes. Scarcely developed mycelia are hidden in these; the development of a gum-like substance in the necrotically changed tissue is characteristic for this interaction type and, for type "0." Mycelia advancing into the mesophyll, and penetrating with their haustoria into cells, are found in reaction type "0" that has been very extensively studied by Allen. After a relatively short period of incubation, "degenerative" changes occur in the invaded cell. The cytoplasm is condensed around the nearest haustorium. The physiological breakdown is indicated by progressive disintegration of plastids and cell nucleus, brown discoloration of cell contents, and swelling of cell membranes. Sharply defined, scorched-looking flecks are the end result. At the same time changes occur in the hyphae that indicate "exhaustion" of the pathogen: the hyphae show an increased affinity for nuclear stains, cell nuclei disintegrate into a homogeneous mass, density of the cytoplasm simultaneously increases in the haustoria-mother cells.

In eusymbiotically interacting H/P combinations (reaction type 4) the interaction fares differently. Degenerative changes in pathogen as well as host occur relatively late, about the time when the pathogen achieves fructification. Interaction types 1 and 3 occupy an intermediate position. In individual cells, necrosis—as far as its strength is concerned—is characteristically subject to a much greater variability in a narrower space than in type 0 or 4.

In principle, a similar course of HR can be observed in other rust diseases, such as flax rust (Hart, 1926), corn rust (Wellensiek, 1927), or bean rust (Wei, 1937). The same is true for downy mildews, e.g., *Plasmopora viticola* (Husfeld, 1931) and *Peronospora manshurica* (Lehman, 1958).

*Erysiphe type:* As far as the available literature shows, mode of interaction and frequency of infection vary here too, one independent of the other (Salmon, 1905). As Salmon already recognized the fate of the pathogen is determined only after the infection peg has penetrated into the cell of the epidermis. According to Neger (1923), who studied the host spectrum of *Erysiphe cichoracearum* and *E. polygoni* isolates, a premature collapse of the invaded epidermal cells occurs. The various H/P combinations seem to react differently. According to White and Baker (1954) and Hirata (1956) it is not the invaded cells but the adjoining mesophyll cells that react with necrosis in cereal mildew. Furthermore, White and Baker established a positive correlation between speed of reaction and degree of resistance. The sooner the collapse of mesophyll cells occurs, the weaker the development of extramatrix mycelium.

*Synchytrium* type: The behavior of potato toward *Synchytrium endobioticum* (Köhler, 1928, 1931) may serve as an example. Again, there is no difference between normo- and hypersensitive types in frequency of infection. It is found only in the degree with which the cell of the host "tolerates" the pathogen. Only meristematic tissue is prone to infection. It is characteristic that the tissue adjoining the infected cells in the eusymbiotic H/P combinations, responds to infection with development of tumors. In case of highest sensitivity the infected cells die after a few hours; so does the pathogen. In the intermediary reaction types, the cell of the host and the pathogen remain alive for some time. But, before there is tumor development in the tissue surrounding the infected cell, necrotic changes set in and lead to a cutting off and expulsion of the infection focus. In a borderline case, a great deal of cell multiplication continues in the neighboring tissue, but here too, abortion of the infection focus occurs, before the sori of the parasite have ripened. The normosensitive or susceptible host type is the opposite of these more or less hypersensitive host types. Here the cell of the host as well as the tissue surrounding the infection focus remains alive. The tissue develops into extensive tumors and the pathogen can multiply.

## 2. Facultative-Biotrophic Fungi

*Phytophthora* type: This type shows, histologically speaking, numerous similarities with the *Puccinia* type. The pertinent examinations have been almost entirely carried out with potato (interspecific hybrids) and *Phytophthora infestans* (Müller, 1931, 1953; Müller and Börger, 1940; Müller *et al.*, 1955; Meyer, 1940; Priston and Gallegly, 1954; Tomiyama, 1955, 1956a, b; Takakuwa and Tomiyama, 1957). *Phytophthora infestans* develops haustoria, as numerous obligate biotrophic parasites do that remain alive a relatively long time in eusymbiotic H/P combinations. Again there is no connection between the reaction type and infection frequency, either. The leaves react to the infection with rapid necrosis. The collapse of the cell of the host precedes the dying off of the pathogen, but its span of life in the necrotic tissue varies in different H/P combinations (Müller, 1953).

In the tubers, the interaction is considerably slower than in the leaves. If the infected cells show signs of an initial physiological breakdown after a 24-hour incubation period (appearance of melanins, regression of turgor, etc.), the pathogen can penetrate only as deeply as a few cell layers into the parenchyma. Under such conditions, no fructification of the fungus takes place. Since both extremes, "highly susceptible" and "highly resistant" are connected by intermediary steps, no alternative separation into "susceptible" and "resistant" is possible in tubers



(Müller, 1935; Müller *et al.*, 1955). In the highly susceptible tubers, the tissue attacked by the pathogen remains alive for weeks and the haustoria of the pathogen exhibit especially strong growth, and the sheaths that surround them reach maximum strength.

*Ustilago tritici* type: In eusymbiotic combinations, systemic infection of the host plant is characteristic for this type. In the first type infection becomes evident only when the host flowers. Such a course of development presumes extensive mutual tolerance between both interaction partners. The other extreme is realized when, as in smut of oats caused by *Ustilago kollerii*, development of the pathogen stops in the first stages of infection. The invaded cells of the host collapse, and the pathogen stops growing simultaneously (Western, 1936). As Oort (1947) and later Király and Lelley (1956) have established, the intermediate degrees of sensitivity are characterized by strong reduction of tillering and longitudinal growth. The intermediary types show greatly changing behavior in clinical results. In some plants the pathogen may grow into the flower region, in others it fails to reach the inflorescence, and the plant remains "free of disease," clinically speaking.

### 3. *Necrotrophic Fungi*

The high degree of mutual tolerance between host and pathogen in the presence of susceptibility is characteristic of the above described interaction types. In the necrotrophic pathogens, on the other hand, there is not a eusymbiotic relationship. If the HR mechanism fails, the pathogen produces a more or less rapidly developing decay of the attacked tissue.

The combination *Pyrus malus* and *Venturia inaequalis* should still be considered as a transition type. According to Nussbaum and Keitt (1938) and Shay and Williams (1956) the highest degree of susceptibility is characterized by a normal appearance of epidermal cells 9 to 10 days after infection by the subcuticular route. The pathogen spreads freely below the cuticle of the host and fructifies before the cells have finally collapsed. On the other hand, the impending physiological breakdown can be foreseen soon after infection in the cells of resistant forms of apples. Infection foci of neighboring cells also show symptoms of an initial necrosis. In the case of highest sensitivity, infection remains limited to a small group of cells, and no more fructification of the fungus takes place. Nussbaum and Keitt describe a third mode of reaction in which the development of a weak mycelium occurs. However, the cells that have come into direct contact with hyphae show no necrotic changes.

In the following H/P combinations, no eusymbiotic relationship,

even a temporary one, seems to occur: *Phaseolus vulgaris* and *Colletotrichum lindemuthianum* (Leach, 1923; Pierson and Walker, 1954), *Cucumis sativus* and *Corynespora cucumerinum* (Klimke, 1941), *Linum usitatissimum* and *Colletotrichum linicolum* (Schwinghamer, 1954), *Hordeum sativum* and *Septoria passerini* (Green and Dickson, 1957), *Hordeum sativum* and *Helminthosporium gramineum* (Skoropad and Arny, 1956), *Zea mays* and *Helminthosporium maydis* (Jennings and Ullstrup, 1957). All these combinations have the factor in common that the pathogen can penetrate into a susceptible as well as a resistant host, and that in case there is resistance, a necrosis progressing rapidly in the infected host cells halts the establishment of a pathogenic relationship prematurely. On the other hand, in the susceptible host, while spreading from the site of infection the pathogen destroys the tissue of the host, often in advance with the dissolution of middle lamellae. Generally, the extremes are connected by intermediary reaction steps. The histological pictures often give the impression that the reaction depends primarily on the speed of the "disintegrating"—i.e., pathogenic—action of the fungus and the speed of the counteraction by HR of the host whether the spreading of the pathogen can be arrested in time.

More often than during attack by biotrophic pathogens, the host develops more or less marked "demarcation tissues" that shut off the infection from the healthy tissues. An instructive example is the shot-hole syndrome, described in detail by Naef-Roth (1948), that is produced in *Prunus* species by infection with *Clasterosporium carpophilum*, and other leaf inhabiting pathogens. Basically, this appears to be a hypersensitivity reaction of intermediary degree, in which the spreading of the pathogen is halted relatively late. Then, approximately 20 cell rows away from the necrotic tissue complexes, the cells begin to divide and bring about the development of a concentric periderm for the separation of the necrotic tissue.

The hypersensitivity reactions produced by leaf pathogens were the first ones to attract the attention of phytopathologists. The hypersensitivity reactions occurring in other organs have only recently become the object of intensive studies. Thus, Flentje (1957) proved for *Pellicularia filamentosa* (= *Rhizoctonia solani*) and Hooker (1956) for *Pythium* types, that the reaction of the invaded cells of resistant hosts is analogous to changes, which have all the earmarks of an HR, histologically speaking.

The examples cited up to now are hypersensitivity reactions that are produced by infection of the host with "adequate" pathogens. The question is, what happens when the spore of a pathogenic fungus lands on the surface of a plant that does not belong to the natural host spectrum of the pathogen? This question was raised as early as 1905 by

Salmon. He found that *Erysiphe graminis* also penetrates into "wrong host plants," but stops growing there after a short initial development. Similar results were obtained by Corner (1935) for other mildew fungi (*Podosphaera leucotricha* or *P. pannosa*). Hori (1935) and Müller (1950) studied the behavior of the causal organism of late blight of potato and tomato, *Phytophthora infestans*, toward plants that are not known as natural hosts of this pathogen (among them Composites, Papilionaceae, and Liliaceae). If the pathogen succeeds in penetrating the tissue of the host, the cells respond with rapid necrosis, and the pathogen simultaneously stops growing. Sproston (1957) obtained similar results with *Monilinia (Sclerotinia) fruticola*, *Alternaria tenuis* and *Botrytis allii* after transfer onto *Impatiens balsaminea*.

### B. Hypersensitivity Reactions Induced by Viruses

If they do not exceed a certain size, parts of tissue that become necrotic after virus infections are generally called "local lesions." Since in many cases no virus can be isolated at some distance from the local lesion, this phenomenon also must be based on a mechanism that localizes the pathogen. The necrotic tissue, however, contains considerable quantities of active virus for some time. According to many authors (e.g., White, 1954; Harrison, 1956), virus synthesis stops when physiological breakdown begins. According to Rappaport and Wildman (1957), the spreading rate of local lesions is constant in certain H/P strain combinations of *Nicotinia glutinosa* and tobacco mosaic virus, so that there is no absolute inhibition of the virus in this instance. Furthermore, a close positive correlation was found between the size of local lesions and quantity of virus that can be separated from the necrotic tissue. This result should point to the lack of an inactivation mechanism. But by using a slowly growing strain, regression of the relative virus quantity was obtained, which indicates that considerable quantities of virus are destroyed or inactivated in the necrotic tissue. Such an inactivation mechanism was postulated by Zech (1952). This author found that the center of older local lesions contains no more active virus, while large quantities of active virus could still be found in the border areas. Thus, in certain H/P combinations, HR could elicit not only localization but partial inactivation of the virus.

As far as the histological changes occurring during interaction are concerned (see Esau, 1938), the following observations should suffice: The first changes recognizable with the naked eye occur in the infected tissue relatively early (Holmes, 1929)—in *Nicotiana glutinosa* and tobacco mosaic virus after 30 hours. According to Bald (see Rappaport and Wildman, 1957) the cell nucleus is affected first, then the chloro-

plasts disintegrate and protoplasmic streaming ceases. Finally, the cell collapses. In cases studied by Ball, a new layer of cells is attacked by this degenerative process, every 4 to 5 hours. The span of time, between infection and dying off of an individual cell, is 6 to 20 hours.

The symptomatic picture of HR occasionally varies greatly, depending on H/P combinations, age of host plant, and external circumstances (see page 490). Considerable differences can occur even in the same organ. In *Nicotiana tabacum* and potato X virus (strain Bf) Köhler (1951) described three local lesion types occurring in the same leaf, that differ in the varied arrangement of the necrotic and still living chlorophyll-carrying tissue rings. These differences might be based on periodic fluctuations of cellular sensitivity, according to Köhler.

As in fungal infections, HR can be chiefly observed when the host spectrum of an individual virus is examined. Schmelzer (1957) studied the reaction of 589 species of plants toward the tobacco rattle virus. Of these, 158 species, almost 25% reacted with local lesions. Of these, many species were not closely related to the *Nicotiana* genus. No dissemination of the virus into the surrounding tissue was observed in all these cases.

### C. Hypersensitivity Reactions Induced by Bacteria

But little information is available to indicate that HR is also involved in bacterial diseases. Such a possibility should not be discarded, however. First, the etiological and the symptomatological momentum has taken precedence in the research of bacterial diseases up to now. Second, in bacterial diseases, if compared to mycoses, the interaction between host and pathogen is much more difficult to observe *in vivo*. However, the examples that follow should indicate that in bacterial diseases too, defense mechanisms occur that can be compared to HR induced by fungal organisms. When inoculated onto inadequate host plants, phytopathogenic bacteria may multiply for the first time, but stop growth after a certain period of incubation. Since no morphological barriers are observed, resistance probably results from physiological incompatibility of host and pathogen (Thiers and Lester, 1949). The fact that many plants respond to bacterial infection with local lesions, indicates a mechanism which has much in common with HR to fungal infection. This idea is supported by the quantitatively different behavior of cotton species toward *Xanthomonas malvacearum*. The leaf lesions are much smaller in the resistant types than in the susceptible ones (Thiers and Lester, 1949). Furthermore, *Pseudomonas tabaci*, elicits leaf blight in tobacco, and the closely related—perhaps even identical—*Ps. angulata* elicits only “angular leaf spot.” Finally, the results by Fuchs *et al.* (1957)



should be mentioned, according to which *Pseudomonas mors-prunorum* (? = *Ps. syringae*) produces "die-back" in the branches of *Prunus* species, and only local necrosis in the leaves. The necrotic tissues are eliminated in the same way as in the shot hole disease of apricots and other stone fruits, caused by *Clasterosporium carpophilum* (see page 477).

#### D. Reactions Resembling Hypersensitivity

Painter (1951) discusses the reason why a plant is unable to serve as a host to a certain insect. Among other things, he mentions hypersensitivity or hypersusceptibility. After giving a number of examples favorable to this concept, he concludes that hypersusceptibility and the resultant apparent resistance is not offered as an explanation for any insect-plant relationship but may be involved, especially in regard to insects with sucking mouth parts.

Studies by Börner (see Börner and Schilder, 1932, 1934) and his colleagues (e.g., Schilder, 1947) on the reaction of resistant *Vitis* spp. and their hybrids with cultured grape varieties susceptible to *Phylloxera vastatrix* can offer the best insight into the problem. The leaf tissue of highly resistant hosts reacts to the attack by the insect with locally limited necrosis; simultaneously, the formation of tumor-like tissue typical of the susceptible host does not develop around the site of the bite. Since the development of a tumor appears to be the prerequisite for normal development of the insect, the plant remains free of damage. Five different overlapping reaction steps are differentiated. There is a positive correlation between the reaction of leaves and of roots, but the latter react to the attack by the insect less violently than the leaves. The sites of the bite are shut off by an inner wound periderm from the remaining tissue. The necrotic tissue parts are later "cast off" during the heavy growth.

A reaction mechanism similar to the one just described is suspected to be the cause of resistance to nematodes (literature quoted by Chitwood and Oteifa, 1952). The dying off of animals in the resistant hosts is ascribed to a premature disintegration of the tissue around the site of penetration, that cuts the animals off from their food supply.

#### III. HYPERSENSITIVITY AS A GENETIC PROBLEM

Biffen (1907) was the first to show that the hereditary resistance of rusts in cereals follows the Mendelian laws. A second, even greater impetus came from Stakman's work (1914, 1915). It was soon found that HR toward individual rust races is controlled by individual genes. Finally, it was possible to clarify the genetics of the parasite's pathogenic

ability in a whole series of H/P combinations. From the wealth of available literature, the following important points should be stressed within the framework of this essay, by which *Triticum vulgare* and *Puccinia graminis*, and *Linum usitatissimum* and *Melampsora lini* can serve as models.

The hypersensitive behavior toward an individual physiological race of the causative agent is usually characterized by a monogenous heredity in which "hypersensitivity" is mostly predominant over "normosensitivity." As a rule, an individual gene controls hypersensitivity toward several races of the causative agent, but various genes can determine hypersensitivity toward the same race. Analogous conditions are found in the pathogen. When this one interacts, as in rust fungi, during the dikaryon phase, "virulence" is chiefly recessive to "avirulence." Here too, a whole series of genes is involved. In the flax rust, at least 25 genes each for the pathogen as well as for the host, are controlling HR. The actions conditioned by individual genes—i.e., reaction potentials—interfere in pairs with each other; thus, the HR can only function when  $R_1$  (resistance of the host) meets  $Av_1$  (avirulence of the pathogen) in the cell of the host, or  $R_2$  with  $Av_2$ , etc. Flor (1955), who has made the most detailed studies of this problem, interprets the situation as "conditioned by specific pairs of genes, one in the host and the other in the pathogen" (gene-for-gene relationship).

If one assumes that dominance corresponds to the building up of a substance and recessiveness to the opposite, then HR is triggered off by the interaction of two specific substances that are adjusted one to the other, one of which originates from the cell of the host, the other of the pathogen. Catcheside (1949) therefore compared the interaction of these two hypothetical substances with the antigen-antibody reaction, as we know it in animal pathology.

Many findings obtained from H/P combinations analyzed in less detail fit easily into this general concept of the genetic basis for HR. Hypersensitivity usually proves to be the dominant trait even in combinations where the pathogen is not an obligate biotrophic organism. It is mostly controlled by a limited number of genes. This is also true for the heredity course of resistance to viruses that is characterized by local lesion reaction. However, dominance of hypersensitivity is not always manifested. According to Holmes (1934) and Weber (1951) and others, in interspecific *Nicotiana* hybrids, for instance, the ability to respond with local lesions to infection by various strains of tobacco mosaic virus is controlled by hereditary factors with an incomplete dominance. Honecker (1934) reports that resistance to mildew is inherited as a recessive trait under low temperatures ( $15^{\circ}$ – $25^{\circ}$  C.) and

as a dominant one at temperatures above 25° C. Such a change of dominance was observed mainly in breeding material that was obtained from crossing susceptible with moderately resistant types of barley. Perhaps the simple gene dose is not sufficient to respond to the attack by the pathogen with a HR when the plants are kept under 15°–25° C. temperature. Müller's (1930) hypothesis was based on a similar conception according to which resistance of potatoes to *Phytophthora* races A and C is based on the cumulative effect of 4 alleles of the R<sub>1</sub> gene and that a certain gene dose is necessary to set an effective HR into motion.

In a whole series of cases, however, the polygenous inheritance of hypersensitivity toward individual races of the pathogen has been obtained; e.g., for interspecific *Vitis* hybrids and *Phylloxera vastatrix* (Börner and Schilder, 1934).

The methods of mutation research have been used to clarify the genetic base of HR, in addition to the genetic analysis of hybrids that were obtained by intra- and interspecific crossings. Freisleben and Lein (1942) obtained mutants of barley, resistant to *Erysiphe graminis*, by X-ray treatment. Bandlow (1951) found that resistance thus obtained is based on hypersensitivity. Analogous results were obtained by Frey and Browning (1955) who produced mutants resistant to stem rust (reaction type 0 to 2). Here again, hypersensitivity proved to be dominant and conditioned by a single gene. On the other hand, Keitt and Boone (1954) succeeded in changing the pathogenic potential of *Venturia inaequalis* populations by treatment with chemical compounds that produce mutations.

#### IV. HYPERSENSITIVITY AS A PHYSIOLOGICAL PROBLEM

Physiological research has contributed doubly to the clarification of the mechanism on which HR is based: first, in clarifying the physiological changes that occur in the parabiologically reacting tissues, and second, by finding out how far external factors influence the interaction between host and pathogen.

##### A. Comparative Physiology of Hyper- and Normosensitive Tissues

Most studies of this problem are concerned with H/P combinations in which an obligate biotrophic organism (including viruses) acts as a pathogen. Indeed, those studies have simply contributed the essentials needed for understanding "obligate parasitism." But, the one-sidedness in the choice of test objects also elicited a one-sidedness in the causal-analytical interpretation of HR. Namely, the physiological changes occurring in the hypersensitive tissues of the host and their extensive and early corresponding inhibition of the pathogen is mainly interpreted

as a simple nutritional relationship. Actually, the relationships are not as simple as that. In any case, an interpretation based on this has to fail when HR is produced by an organism which can be grown on quite a variety of artificial nutrient media.

Brown (1955) characterizes the situation as follows: "To say that *Puccinia graminis* cannot progress through dead tissue is a truism if one accepts the current definition of an obligate parasite, but it is by no means excluded that the agent which inhibits the growth of *P. graminis* on an unsuitable host is comparable to that which acts similarly on *Phytophthora infestans* in the cells of a resistant potato variety. At all events the various responses of potato tissue to attack by the blight fungus are much more open to analysis than are the responses of wheat to the rust fungus and so, as it is good practice in ascending a staircase to begin on the bottom step and from there to work upwards, the same procedure could well be applied to the study of parasitism."

### 1. Time Relationships

One of the essential characteristics of HR is the relative rapidity with which the cell of the host undergoes necrotic changes in response to infection with the avirulent pathogen. The span of time between infection and final breakdown of the cells of the host varies, depending on the underlying H/P combination and external circumstances; in broad terms, however, as far as more detailed studies show, it is always shorter in parabiologically interacting H/P pairs than in the eusymbiotic ones. In potato (Ackersegen type) and *Synchytrium endobioticum*, for instance, collapse of the cell is observed after a few hours (Köhler, 1931); the interaction is finished after 16 to 18 hours in the combination *Phaseolus vulgaris* and *Phytophthora infestans*, to be discussed in more detail in Section IV. In rust infections, on the other hand, days pass before there is a complete breakdown of cells of the host in parabiologically interacting H/P pairs. However, it takes place much more rapidly than in eusymbiotic H/P combinations.

The most detailed quantitative studies of rate of interaction and behavior of resistance are available for potato and *Phytophthora infestans*. According to Meyer (1940) and Müller and Börger (1940), at least five phases can be distinguished in the morphological and physiological changes that can be observed in the parenchymal cells of tubers.\* The parabiological combinations go through these five phases much faster than the eusymbiotic ones, the extreme being 36 hours (19° C.). In eusymbiotic combinations, on the other hand, the infected cells remain alive up to 3 weeks and the whole tuber is destroyed. Between these

\* See also Gäumann (1948).



extremes are types of medium interaction rate and resistance. There is a clear relationship between both characters: the earlier the first signs of necrosis are observed, the earlier the pathogen stops growth. In extreme cases the area of tissue that has been penetrated by the pathogen is limited to a few cells (Müller, 1953).

Ferris (1955) did similar studies on the HR in leaves. The differences between the resistant and susceptible plants, as far as the rate of reaction is concerned, were not so striking. But she found that "a necrotic response was evident in the resistant plants at most a few hours before such a response developed in susceptible plants." The first differences in the mode of reaction between sensitive and normosensitive tissues become evident almost immediately after the pathogen has penetrated into the tissue of the host. Thus, increased plasma current and Brownian molecular movement start in the hypersensitive cells 10 to 60 minutes after infection, in the normosensitive cells only after 120 to 180 minutes (Tomiya, 1956b). Analogous, but not as great, differences were observed in the speed of necrotic breakdown (Priston and Gallegly, 1954; Tomiya, 1955, 1956a).

The concept of reaction rate as a deciding factor of HR only makes sense when we assume that during interaction the living conditions for the pathogen "become worse." Unfortunately, we are informed only incompletely about the chemical-physiological side of necrosis. But the substantial changes occurring during HR have to differ from those that occur for instance during necrosis induced by wilting toxins. Otherwise one could not understand why common saprophytes, such as *Alternaria tenuis* in *Impatiens balsamina*, discontinue their growth shortly after having penetrated into the tissue of the host.

## 2. Changes in Chemical Constituents

An integrating symptom of HR is the rapid loss of turgor in the infected cells. The water released by them is mostly absorbed by neighboring living cells. Consequently, the latter swell and fill the space left by the dead ones. Evidently, substances are also transferred which produce characteristic reactions in the neighboring tissue, such as wound periderm buildup, swelling and migration of cell nuclei toward the cell wall bordering upon the necrotic cell, or vacuolization of cytoplasm. Before the final physiological breakdown of the cell of the host, the hydrogen ion concentration increases in the cell (e.g., Müller, 1958a). It is characteristic of some parabiotic H/P combinations that the amylolytic enzyme system is put out of commission; e.g., in potato tubers and *Phytophthora infestans* (Meyer, 1940). In the eusymbiotic combinations, on the other hand, a considerable decomposition of starch occurs in the infected tissue (Lepik, 1930).

Tomiyama *et al.* (1956b) studied the water, carbohydrate, and protein content of eusymbiotic and parabiatic H/P combinations in potato tubers infected by *Phytophthora*. They found in the latter ones—contrary to the eusymbiotic combinations—during the early stages of infection an increase in the water-soluble protein portion, in starch, and in content of phenolic compounds. The situation changed later on. The writer concluded that the infection results in the synthetic reaction in the tissue of resistant varieties. This is a nonspecific reaction of the host tissue to the damage done by the pathogen.

According to Noll (1950), a great accumulation of silicic acid (magnesium silicate?) occurs at the site of infection in parabiatic combinations (reaction type 0) between wheat and *Puccinia glumarum*.

The reaction of infected cells to stains is different in parabiatically and eusymbiotically reacting tissues. Irreversible affinity of cell wall and cytoplasm for basic vital stains (e.g., rhodamine B or neutral red, 2 p.p.m.) occurs much earlier in parabiatically than in eusymbiotically reacting tissue complexes. The individual cell shows increased affinity for the stain first where it is in direct contact with intercellular hyphae or haustoria of the pathogen. Thus, the cell does not react as a whole to the infection (Meyer, 1940).

Unfortunately, there is not much information about the chemical nature of brown pigments, the most marked symptom of HR next to cell collapse. Undoubtedly the pigments contain phenol groups (Dufrenoy, 1936). According to Meyer (1940) they are deposited as phlobaphenes mainly in the intercellular spaces of the cell walls. Since the cell membranes react positively in advancing necrosis to phloroglucin-hydrochloric acid, some authors think there is also lignification.

Buildup of tannin-like substance often precedes the appearance of brown pigments. Since these have antibiotic qualities, some authors consider them "antibodies." Dufrenoy (1936) tried to clarify the immunological significance of this group of substances. Humphrey and Dufrenoy (1944) postulated that in *Avena sativa* and *Puccinia coronata* there is a causal relationship between coacervation of phenolic compounds at the site of infection and the "decomposition" of the respiratory system. When this is so great "as to prove rapidly lethal, the rust fungus no longer behaves as a parasite, but as a pathogen inducing necrotic spots characteristic of 'hypersusceptibility'."

### 3. Metabolic-Physiological Changes

a. *Respiration*: As is generally known, an increase in the intensity of respiration occurs in the parasitized tissues, that is attributed by Allen and Goddard (1938) mostly to higher respiratory activity of the tissue of the host. Recently, attention has been drawn to the respiratory

metabolism of parabiologically interacting H/P combinations as well. Attempts have been made to gain insight in two ways: First, by comparative studies in eusymbiotic and parabiological H/P combinations of respiratory intensity or of the activity of respiratory enzymes separated from host tissue and, second, by studying whether through manipulation of external circumstances or treatment of tissue with respiratory inhibitors the reaction potential can be changed according to a proposed working hypothesis.

According to Millerd and Scott (1956), in parabiological combinations of *Hordeum sativum* and *Erysiphe graminis* respiration is clearly increased at the beginning of interaction, but the respiratory intensity falls sharply again, and the growth of the pathogen stops simultaneously. In eusymbiotic combinations, on the other hand, the respiratory intensity was clearly higher than in the control plants, even 168 hours after infection. Hosts with intermediary resistance behavior showed an intermediary character in the respiration behavior. Samborski and Shaw (1956) and Shaw and Samborski (1957) found a similar relationship in wheats and *Puccinia graminis*. Japanese workers (e.g., Tomiyama *et al.*, 1956b) have stressed that respiratory increase is an essential factor in HR.

Millerd and Scott (1955) reported that from extracts of leaves attacked by mildew they had isolated a heat stable dialyzable factor, which effects an increase in respiratory activity of healthy wheat leaves. Later, a phenolic compound was isolated from the raw extracts, which produces increased sensitivity toward the mildew fungus in susceptible plants.

Sempio (1950a, b) tackled his studies on the influence of light and other external factors on the resistance of wheat to *Erysiphe graminis* from a "metabolic resistance" point of view. This metabolic resistance is determined by the intensity of the photosynthetic, glycolytic, and respiratory activity of the host. According to him, the anabolic processes dominate over the catabolic processes in plants that are in a condition of defense, while the opposite is true for plants that are in a condition of susceptibility. Unfortunately, Sempio does not report, how far the interaction *type* can be changed by the manipulation of the environmental conditions. Therefore, it is difficult to determine how applicable to HR is his broad generalization that an unbalanced increase in the respiratory rate is always linked with a marked decrease in the metabolic defense.

The following studies have also made clear that there is a strong connection between the HR mechanism and respiratory metabolism. If potato tubers that normally interact parabiologically with certain races of *Phytophthora infestans* are treated with respiratory inhibitors such

as pyrocatechol or tyrosine, the reaction will shift to the eusymbiotic type. The same effect appears after pretreatment of tubers with polyphenoloxidase inhibiting compounds (e.g., phenol urethane or potassium cyanide) or with acids from the Krebs cycle (Fuchs and Kottc, 1954; Christiansen-Weniger, 1955). Király and Farkas (1957) found that in all five types of wheat, of which two interacted eusymbiotically and three parabiotically with the *Puccinia graminis* race they used, the  $O_2$  uptake was clearly higher than in the controls, after 7 to 10 days incubation. However, quantitative differences between the different reaction types were found. In eusymbiotic combinations,  $O_2$  uptake was 6 to 12 times higher than in the parabirotic ones. On the other hand, the glycolic acid oxidase activity decreased in all interaction types, more in the parabirotic than in the eusymbiotic combinations. The decrease of glycolic acid oxidase is explained by the authors with the assumption that the prosthetic group of the enzyme (lactoflavinephosphate) is in great part withdrawn from the cell of the host by the vitamin hungry pathogen. The greater decrease of the enzyme activity in parabirotic combinations can be explained by the fact that an additional inactivation of the enzyme occurs in the dying tissues.

Another interesting attempt to gain a glimpse into the dynamics of energy metabolism of eusymbiotic and parabirotic H/P complexes (rust and mildew in cereals) was undertaken by Shaw and Hawkins (1958) who pursued the decarboxylation of artificially produced indoleacetic acid (labeled with  $C^{14}$ ). A sharply increasing elimination of  $C^{14}O_2$ , soon followed by a sharp decline, was found in both combinations.

It is impossible at this time to form a coherent—even hypothetical—opinion of the respiratory-physiological basis for HR. One thing is certain. There are basic quantitative differences underlying the eusymbiotic and parabirotic interactions. This is indicated by two facts: in the first stages of interaction *both* types show a marked increase in the respiration rate as compared to controls; also, as far as the external course of HR is concerned, the behavior of intermediary interaction types can be shifted by manipulating external conditions toward *both* sides (see page 491). As would be expected, there is a sharp drop in respiration rate after a certain incubation period in parabirotic H/P combinations—contrary to the eusymbiotic ones—(established by Millerd and Scott, 1956, and other authors), because the growth of the pathogen as well as metabolic activity of the host tissue stop after a short incubation period. The only thing we can say today is that in parabirotically reacting cells the intensification and the diversion of the respiratory metabolism induced by the pathogen surpass the proportion of eusymbiotically reacting cells, consequently, the premature physiological breakdown of the



host cell and the inhibition of the pathogen are connected with an especially high show of energy by the reacting cell of the host (see Farkas and Király, 1958).

b. *Photosynthesis*. No special discussion is needed for the fact that assimilation activity ceases in the parabiotically reacting tissues. In this connection, studies by Gassner and Goeze (1936) show that almost no decrease of assimilation activity occurred in reaction type "i" of wheat infected with yellow rust, but a reduction of more than 50% in reaction types "0 to 2" and "4."

c. *Permeability Relationships*. For the maintenance of a eusymbiotic relationship there must be a physiological balance for a time between the interfaces of host and pathogen, where they are in direct contact, so that a metabolic exchange can take place between both partners. According to present knowledge, this exchange appears to occur chiefly by osmosis. According to Meyer (1940), semipermeability is maintained 6 to 14 days (19° C.) in the parenchyma of potato tubers infected with an eusymbiotic strain of *Phytophthora infestans*, while it is lost after 34 to 48 hours in parabiotic interaction. Thatcher (1942) used the rate of deplasmolysis as a measuring stick for permeability of plasma membranes and found in rust-infected wheat that permeability increases in eusymbiotic combinations; in parabiotic combinations, on the other hand, it decreases in the cells adjoining the necrotic tissue. Thatcher postulated that any factor altering the permeability of plasma membranes of a particular host tends to modify its susceptibility, provided that other changes in the host-pathogen relationship arising from the same cause are insufficient to offset this susceptibility change. This conclusion could go too far, since Thatcher's results only indicate that in parabiotic combinations a change in permeability occurs in advance of the hyphal tips, and physiological changes in advance are usual for HR. For instance, chlorotic fadings are characteristic in the tissue immediately around the area attacked by the pathogen; chlorosis is obviously due to substances that diffuse from the necrotic tissue into the adjoining cells.

Greenham and Müller (1956) used the ability of tissue to conduct electricity in potato tubers infected by *Phytophthora infestans* for quantitative determination of the damage suffered by the cells of the host in eusymbiotic and parabiotic H/P complexes. Osterhout's classic theory is that at low frequency, living tissues have low conductance, because the cell membrane is impermeable to ions but the permeability may be increased by the action of poisons. According to expectation, the conductance ability increased clearly 26 hours after infection in the parabiotic combinations, but it did not change after still another 24 hours in eusymbiotic combinations. After prolonged waiting, it increased in para-

biotic combinations 25 cell diameters away from the infection focus. This "in advance effect" was missing in eusymbiotic combinations. In the latter case, the increase in conductance ability was found only at a distance of 51 cells *behind* the foremost hyphal tips.

d. *Studies with Isotope Labeled Compounds.* Results obtained with the use of isotope technique are summarized as follows (see Shaw *et al.*, 1954 and Shaw and Samborski, 1956; Yarwood and Jacobsen, 1955). In eusymbiotic combinations, e.g., *Phaseolus vulgaris* and *Uromyces appendiculatus*, *Helianthus annuus*, and *Puccinia helianthi*, and others, a strong accumulation of labeled compounds occurs ( $C^{14}O_2$ ,  $H_3P^{32}O_4$ ,  $H_2S^{35}$  and a series of organic compounds with labeled carbon). On the other hand, accumulation is weak or absent when labeled compounds are fed to parabiotic combinations. Since the accumulation is strongly reduced, by cutting off oxygen or by pretreatment of tissues with DPN, this phenomenon must be connected with the energy metabolism of the tissue of the host. Accumulation of isotope labeled compounds is never observed in tissues that had been attacked by necrotrophic pathogens such as *Venturia pyrina*, *Septoria aesculi*, and others. Shaw *et al.* (1954) formulated this thought about obligate parasitism. A prerequisite for normal development of biotrophic parasites is sufficient supply of substrate to the infected tissue, from the outside. This process depends on the physiological condition, especially on the respiration of the infected tissue. If the tissue of the host is killed prematurely by the pathogen, however, it is plausible to assume that the accumulation of isotope labeled compounds stops too.

e. *Quality, Position, and Age as Interfering Factors.* The capacity to respond to infection with HR can be quite different in different tissues of one and the same individual. In barley plants, resistant to *Helminthosporium gramineum*, coleorrhiza tissue reacts with "necrotic pockets," while the roots "tolerate" the pathogen and are destroyed by it (Skoropad and Arny, 1956). The organotropically bound sensitivity is especially evident in potato and *Phytophthora infestans* (Müller, 1953; Müller *et al.*, 1955). In certain carriers of gene  $R_1$ , the reaction of the leaves is to limit necrosis greatly. The parenchyma of the tubers, on the other hand, "tolerates" the invasion of the pathogen and the tubers are completely destroyed by it. The marrow parenchyma of the stem behaves in intermediary fashion. The petals—whether the plant carries gene  $R_1$  or not—are always attacked in a normal way. Thus, there is a sensitivity gradient that is characterized by the following successive decrease in sensitivity: leaves, marrow parenchyma, tuber parenchyma and petals. The steepness of this gradient is controlled genetically and one wonders whether "minor genes" or only differentiating "effective doses" of gene  $R_1$  are involved.

The ontogenetic variability of sensitivity is seen even in individual organs. The etiolated tuber sprouts of many *Phytophthora* resistant potato types are less sensitive at the tip than at the base (Müller *et al.*, 1955). This seems generally true for meristematic tissue. On the other hand, "loci of different sensitivity" can be differentiated in the parenchyma of grown potato tubers. In parts of tissue with higher reaction rate, the pathogen stops growth relatively early (Müller, 1953).

The shift of reaction potential that appears during individual development of the plant and its organs, might be closely related to those organo- or histotopically bound sensitivity differences. In half-grown wheat plants, for instance, the leaves react differently to infection with *Puccinia triticina* (Newton and Johnson, 1943), depending on their position on the stalk. Sensitivity decreases toward the base.

The shifting of reaction position with progressive development of the plant in rust diseases of cereals is of special interest. Although the reported findings do not offer a completely uniform picture, it seems that sensitivity usually increases during individual development of the plant (e.g., Stakman and Piemeisel, 1917; Straib, 1940; Newton and Johnson, 1943; Simons, 1954). It has also been established that in virus diseases sensitivity increases with progressive development of the plant (e.g., Holmes, 1932: *Salanum melongena* and tobacco mosaic virus). However, the opposite tendency is also observed, for instance in *Phaseolus vulgaris* and *Uromyces phaseoli* (Wei, 1937).

### B. The Influence of External Factors on the Course of the Hypersensitivity Reaction

The effect of external factors on HR may be a direct or an indirect one. In the case of the latter, predisposition is changed. This problem is discussed in detail by Yarwood in Chapter 14 of this volume. Literature, accumulated since Ward's (1902b) time—chiefly in the field of cereal rust research—is so abundant that the following references have to suffice:

#### 1. Effect of Temperature

The speed with which HR expires as in every living process is largely determined by temperature. Also the degree of sensitivity and the clinical result of interaction usually depend on temperature. Thus, sensitivity increases in wheats and *Puccinia glumarum* as the temperature increases; however, in wheats and *Puccinia graminis*, sensitivity decreases (Gassner and Straib, 1930b; Straib, 1940; Johnson, 1931). Johnson studied the behavior of a whole series of physiological races of *P. graminis* on standard wheat types and found that the proportion of

temperature influence is co-determined by the individual H/P complex. Intermediary interaction types usually show the greatest lability. Among others, Straib (1940), showed for yellow rust and Hayden (1956) for black rust, that the age of the plant has a bearing here too.

Similar relationships have been obtained in other diseases elicited by fungal pathogens. Thus, the mildew-resistant wheat type "Hope," does not give the defense necrosis reaction at temperatures of 24° and 28° C.; that is, it is susceptible at these temperatures (Futrell and Dickson, 1954). The reaction of *Phytophthora* susceptible potato tubers is shifted toward the parabiotic side by lowering the temperature below the 10° C. limit (Müller and Griesinger, 1942).

In virus diseases the temperature also exerts a decisive influence on the interaction between host and parasite, in many cases. The interaction between *Nicotiana glutinosa* and tobacco mosaic virus should be mentioned, as an exemplary case. In temperatures below 28° C. the plants react with "local lesions"; under higher temperatures (35° C.) with systemic chlorosis (Samuel, 1931). According to Holmes (1932), local lesions are much smaller at 16 to 18° C. than at 20 to 25° C. At 10° C. lesions do not develop.

The predisposition of the plant is also influenced by temperature. According to Gradinaroff (1943), under prolonged sublethal pretreatment at temperatures above 35° C., the potato tuber loses its ability to localize infections with the otherwise avirulent *Fusarium* species. Straib and Noll (1944) found that in wheat varieties more or less resistant to *Puccinia triticina* sensitivity is reduced by pretreatment at 50° C. for 60 seconds. Thus, in the highly resistant Malakoff varieties, the reaction type changed from "i" to "2 to 3." However, the leaves regained their high sensitivity if the plants were returned to normal temperatures for 4 days. Similar results were obtained in pods of *Phaseolus vulgaris* which had been treated with 44° C. for 2 hours and inoculated with *Sclerotinia fructicola* or *Botrytis cinerea* (Müller, 1956; Jerome and Müller, 1958).

In virus diseases, too, a gradual decrease of the sensitivity after treatment with supramaximal temperatures, occurs now and then. Thus, if leaves of *Nicotiana glutinosa* are exposed for 40 seconds, to 50° C., and then infected with tobacco mosaic, the diameter of lesions, becomes about 80% greater than in controls, according to Yarwood (1958); on the other hand, the time for lesions to develop decreases.

## 2. Effect of Light and Atmospheric CO<sub>2</sub> Content

In cereal rusts, as a rule, optimal light supply promotes development of the pathogen (Johnson, 1931; Hassebrauk, 1940; also see literature cited by Gassner and Straib, 1930a). At any rate, available litera-



ture does not always make it clear whether inhibition of the pathogen under inadequate lighting is due to increase of sensitivity, or to a "hunger crisis" of the fungus due to insufficient nutrition (Sempio, 1939, 1950b). Bever (1934) varied the day length in barley and *Puccinia glumarum* under average temperature of 9° C. With 15-hourly or continuous exposure to light a highly susceptible variety, "Pasier," showed reaction type "0," estimated only after lack of sporulation of the pathogen. In using lettuce plants the stems of which reacted with necrotic lesions to individual *Pellicularia* strains, Flentje (1957) found that the hypersensitive reaction of the host was lessened by exposure to reduced light intensity. On the other hand, Hassebrauk (1940) succeeded in shifting the reaction type toward increased sensitivity in wheats and *Puccinia triticea* by keeping the plants for two days in darkness during the incubation period. The proportion of induced changes and the time of treatment at which the response was greatest, was different in the different H/P combinations.

Our information on the influence of CO<sub>2</sub> content of the air is limited to the rust diseases. Gassner and Straib (1930a, see also their listed bibliography) are responsible for most detailed studies of this problem. Insufficient CO<sub>2</sub> supply primarily lowers the frequency of infection in yellow rust of wheat, but does not shift the degree of sensitivity. In concentrations above 1.5% CO<sub>2</sub> in the air, changes do occur in the infected host tissues that resemble the rust type 0. "Necrotic discoloration" increases considerably as the CO<sub>2</sub> content increases further; borderline concentrations, where the eruption of pustules in brown rust of wheat is prevented, are not equally high in different interaction types, but are lower in intermediary interacting H/P combinations than in the susceptible ones.

### 3. Influence of Mineral Salt and Water Supply

Because there was hope of obtaining with nutritional-physiological experimentation an insight into the mechanism that prevents normal development of the pathogen in the hypersensitive tissues, special attention was given to the relationships between nutrition of the plant and its immunological behavior (see bibliography in Gassner and Hassebrauk, 1931, 1933; Gassner and Franke, 1934). Since this point will be treated in more detail elsewhere, the following remarks should suffice: As a rule, only hosts with intermediary sensitivity respond to differences in mineral salt supply. Generally speaking—again in cereal rusts—it was found that lack of nitrogen increases sensitivity, surplus of nitrogen decreases it. Potassium exerts an antagonistic influence. The significance of phosphorus could not be unequivocally clarified. Obviously it depends

on the simultaneous supply of nitrogen and potassium. Development of obligate biotrophic parasites is better if the host's growth is greater (e.g., Pantanelli, 1921). Unfortunately, many studies do not differentiate sharply enough between the reaction type and the frequency of infection per unit area of the surfaces exposed to the parasite. The many discrepancies in the literature can be explained from that and from the use of extreme eusymbiotic or parabiatic H/P combinations. This is especially true in cases where the effect of mineral salt nutrition was studied on the reaction to diseases elicited by facultative biotrophic or necrotrophic pathogens (e.g., potato and *Phytophthora infestans*).

Chessin and Scott (1955) found that quantitative differences in the size of local lesions in *Nicotiana glutinosa* infected by tobacco mosaic virus depend on mineral salt supply. Lack of iron or sulfur causes an increase in size of local lesions. This does not happen in calcium or magnesium deficient plants.

In a bacterial disease (*Zea mays* and *Phytophthora stevensii*) seedlings insufficiently supplied with nitrogen reportedly showed "small necrotic lesions but little or no wilting of the invaded leaves" (Spencer and McNew, 1938). This relationship confirms the opinion that in bacterial diseases, too, interactions appear to set in that can be compared to the hypersensitivity reactions so often observed in mycoses.

As far as water supply of the plant is concerned, available information suggests that a low water content increases the sensitivity of tissues. If the plants that have become hypersensitive through wilting are brought back into normal conditions, they resume a normal behavior (Doak, 1930).

#### 4. Effect of Narcotics

Stakman (1914) was the first to work on the influence of gaseous narcotics on degree of sensitivity. He found that treatment of plants with chloroform made an immune plant somewhat susceptible to rust. Gassner and Hassebrauk (1938) confirmed Stakman's results in a whole series of cereal rusts. In certain combinations they succeeded in changing the reaction type from 0 to 4. In potato and *Phytophthora infestans*, treatment of hypersensitive tubers with sublethal concentrations of alcohol provoked a decrease of reaction rate; simultaneously the pathogen was able to penetrate deeper into the tuber parenchyma and fructify (Behr, 1949; Müller and Behr, 1949). Tomiyama *et al.* (1956a, 1957) who confirmed these findings assume that the effect of narcosis to decrease sensitivity is connected with the respiration (dehydrogenase) system of the host.

According to Volk (1931) and Minkevicius (1932) the virulence of

necrotrophic fungal pathogens, e.g., *Alternaria brassicae* on *Brassica oleracea*, clearly increases by pretreatment of the host with chloroform.

### 5. Effect of Chemotherapeutants

Gassner and Hassebrauk (1936) were the first to increase sensitivity and with it resistance of cereals toward rust with the help of a systemic "fungicide," by temporarily transferring young plants into nutrient solutions containing sulfides. The reaction type was thus lowered from 3 or 4 to "0" and "1." The same effect was obtained by Hassebrauk (1951) with sulfonamides and sulfones, which he mixed into the soil. He explains the effect of these compounds as a separation of the structurally similar and for some fungi essential *p*-aminobenzoic acid from its protein carrier, just as in human medicine. Hotson (1953), reported still other organic compounds that are effective against stem rust. In accordance with Hassebrauk's assumption, Hotson reversed the rust inhibiting effect of sulfadiazine by using *p*-aminobenzoic acid.

According to Sempio (1942), cadmium—used as  $\text{Cd}(\text{NO}_3)_2$ —acts as a systemic fungicide against wheat mildew. Meyer (1951) explains the effect of cadmium as an increase in sensitivity of epidermal cells of the host toward the pathogen.

Also, antibiotics applied systemically to the plant can increase sensitivity, at least as far as external symptoms are concerned. The effect of streptomycin in potato and tomato against *Phytophthora infestans* (Müller *et al.*, 1954) is explained by Vörös *et al.* (1957) as a streptomycin induced increase of polyphenolase activity. But "the antibiotic was shown to be totally inactive when tried directly as a 'substrate' in the assay of polyphenolase activity"; thus, the streptomycin effect must be an indirect one. Chemotherapy is discussed further in Chapter 15 of this volume.

### 6. Effect of Irradiation

Norell (1954) showed, that UV-irradiation lowers the resistance of potato tubers to *Fusarium* species, whereby the marrow parenchyma responds better to treatment than the peripheral tissues. X-ray treatment of young wheat and oat plants decreases the sensitivity of intermediary interacting H/P complexes to *Puccinia graminis*; it is unsuccessful in extremely susceptible or resistant hosts. In postinfection treatment, on the other hand, resistance of flax against *Melampsora lini* increases; but only seemingly, because as Schwinghamer (1957) showed, the increase in resistance is based on direct damage to the pathogen by irradiation.

In retrospect, it can be said that the mechanism of HR, that is primarily determined by hereditary factors in the host as well as in

the pathogen is subject, within certain limits, to changes in external conditions and to those of ontogenetic nature. The influence that temperature exerts on the course of HR is especially informative. It not only determines the rate of interaction but also the "biological balance" between the interacting partners. Furthermore, we have seen that the living cell can "repair" inactivation of the HR mechanism caused by supramaximal temperatures. This shows that the ability of the host to respond to the attack of a pathogen with HR is inseparably bound with a certain physiological condition of the cell of the host. If we use the term "*Fließgleichgewicht*,"\* borrowed from biochemistry, and correspondingly presume that the anabolic and catabolic processes are in equilibrium in the uninfected cell, HR can be interpreted as the result of a deviation of this equilibrium induced by the pathogen. This deviation would then be the cause for the premature death of both interacting partners. At any rate, this does not mean much as long as we don't know more about the mode of action of metabolites of the pathogen that induce HR, and about the nature of the factor that prevents further growth of the pathogen in the hypersensitive tissues.

#### V. HYPERSENSITIVITY REACTION AS AN IMMUNOLOGICAL PROBLEM

The main problem here is as follows: What is the nature of the factor that brings about early death in the development of the pathogen in the parabiotic H/P complex? This question was raised earlier by Ward (1902a). Although he was unable to prove the existence of such a factor, he summarized his concept, mainly based on histological findings, as follows: The antagonism between host and pathogen "must be due to something far more subtle than a mere soluble poison oozing from the cells."

Since Ward's day, this question has been discussed in many works and opinions about the antipathogenic principles differ widely. According to many authors, nutritional physiological factors which determine the suitability of host tissue as nutritional substrate for the pathogen, govern whether a typical pathogenic relationship occurs or not. Other authors say that inhibition of the pathogen is due to preformed inhibitors. Since the finely adjusted relationship between pathogen and host, e.g., rust diseases, can be explained by neither supposition, not so much the absolute content of specific nutritional products or specific inhibitors, but the relationship between these two has been suggested as being involved in the establishment of a pathogenic relationship (see for example Garber, 1956; Lewis, 1957). Other authors have concluded,

\* Identical with "stationary state."



mainly on the basis of histological findings, that postinfection chemical changes of the host tissue hinder the normal development of the pathogen. Finally, to reconcile the two seemingly contradictory hypotheses it has been assumed that some substances diffuse from the pathogen that is dying because of lack of nutrition, into the cell of the host and its surrounding; these substances elicit the physiological breakdown of the host cell and thus cut the parasite off from its sources of nutrition.

*A. Lack of Nutritional or Growth Factors as Antipathogenic Principle?*

This assumption stems from the thought that corresponding to the specialization of the pathogen on its host, there is a notable specificity in the nutritional requirements of the pathogen as well as in the chemical make-up of the host. Hereby, all H/P combinations involving necrotrophic pathogens are *a priori* excluded from consideration.

The simplest way of investigating this thesis is to compare the pressed out juices of closely related hyper- and normosensitive hosts with respect to their adequacy as nutritional substrates for the given pathogen. All attempts of this kind have failed until now. Yet, many authors still adhere to the "nutritional hypothesis." The most important arguments for this are: (1) Lack of success in all attempts to find an inhibitor in the hypersensitive host that is absent in the normosensitive one—irrespective of whether it is preformed or is formed after infection; and (2) the obligate biotrophic character of the pathogen in question.

The structures of the specific proteins of the host, and their precursors, as well as of the carbohydrates have been invoked to explain the varied behavior of the host plant toward a certain pathogen or one of its races. Leach (1919) and Wellensiek (1927) were determined followers of this hypothesis. Fischer and Gäumann (1929) used it to explain the extreme specialization of physiologic races of a rust fungus for specific host types. The specific structure of proteins was most often used as an example.

Gassner and Franke (1934) also felt this way; they found that in cereal rusts, the infection type increases with the protein content, under differentiated potassium and abundant nitrogen supply. Since, however, no correlation could be proven between varietal resistance and protein values, they postulated that qualitative differences within the existing protein compounds are coordinated with the hypersensitivity connected with the individual H/P complex. Finally, Johnson's (1953) physiological interpretation of the action of the genes that control HR in cereal and flax rusts is also based on a purely nutritional concept. According to him, genes that control HR are "enzyme producers" and an individual

rust race could function as a pathogen only when it finds the enzymatic system needed for successful parasitism in the host. This purely hypothetical concept, however, does not agree with the interpretation according to which the production of a certain enzyme is bound directly to the dominant allele of the gene.

Attempts have also been made to find correlations between carbohydrate content (mainly of sugars) and varietal differences in hypersensitivity. These attempts can be traced to Comes (1913) who claimed to have found a positive correlation between sugar content and susceptibility (see also Pantanelli, 1921). Even with the use of modern methods for separation of various types of sugar (glucose, fructose, and maltose) no difference could be found in the sugar content among various reaction types of wheats and *Puccinia triticina* (Hassebrauk and Kaul, 1957).

### B. Preformed Inhibitors as Antipathogenic Principles?

It is quite natural that the demonstration of the preformed inhibitors as a cause of inhibition of pathogens in hypersensitive tissues can only be considered successful when the following presumptions are fulfilled: (1) There should be no possibility that inhibitors separated from the host tissue are preparative artifacts; (2) it should be proven that the *in vivo* concentration of the factor in question is sufficient to bring about death of the pathogen in a short time; (3) there should be a positive correlation between the *in vivo* concentration and the sensitivity degree that the host exhibits toward the given pathogen.

Since it has been impossible yet to show that the preformed inhibitors meet these requirements (compare with Virtanen *et al.*, 1956; Valle, 1957; and others) the following condensed survey of studies done on this problem should be sufficient.

#### 1. Acid Content and Hydrogen Ion Concentration of the Host Tissue

Comes (1913) was the first to suggest that acidity of the cell juice plays an important role in the resistance of the plant to its potential pathogen. His results, however, could not be confirmed by later authors (e.g., Hurd, 1924; Hursh, 1924; Newton *et al.*, 1929)—not even when hydrogen ion concentration was used as measure of acidity.

Hassebrauk and Kaul (1957) studied the content of ascorbic, citric, oxalic, and malic acids in extracted juices of wheat species that behave differently toward *Puccinia triticina*. No connections could be proven between acid content and resistance. In wheats and *Puccinia graminis*, Pilgrim and Futrell (1957) achieved the same result for ascorbic acid.

#### 2. Content of Phenolic Compounds in Tissue of the Host

The thought that preformed toxins, particularly of phenolic com-

pounds, could cause inhibition of the parasite in hypersensitive tissues, found many followers, since Angell *et al.* (1930) proved in *Allium cepa* and *Colletotricum circinans* that the relative resistance of brown skinned bulbs is due to the antibiotic effect of phenolic compounds (protocatechuic acid, catechol) that diffuse from the dead exterior scales into the surrounding area and thus protect the inner scales from attack by the pathogen. Dufrenoy's findings (see page 485) according to which there is an accumulation of phenolic compounds in infected tissues, also encouraged further search for preformed substances that have a toxic effect on fungal and bacterial pathogens. But the result of these attempts was disappointing. Although considerable quantities of phenolic compounds could be found in the living tissues, no significant differences could be obtained between the phenol content of hyper- and normo-sensitive hosts (see Newton and Anderson, 1929; Siebs, 1955; Cruickshank and Swain, 1956; Scott *et al.*, 1957). Only in the case of wheat varieties resistant to *Puccinia triticina* did the pressed out juice have a higher content of protocatechuic acid than the susceptible ones (Kargopolova, 1937).

Notwithstanding these discouraging results, many authors still maintain that preformed phenolic compounds somehow have a causal connection with HR. Newton and Anderson (1929) and Scott *et al.* (1957) suppose that in a parabiotic H/P complex activation of the phenolic compounds occurs after the infection. In eusymbiotic H/P complexes semi-permeability of cells is maintained for some time, the phenolic compounds are therefore held back in the cell of the host and thus cannot be effective; in parabiotic combinations, on the other hand, they are "set free" by the dying cells and they can then develop their anti-pathogenic effect. This hypothesis actually presumes that the phenolic substances found *in vitro* are also present *in vivo*, in concentrations that are sufficient to stop the growth of the pathogen. No quantitative analyses on this are available as yet.

### 3. Enzymatic Activity of the Host Tissue and Hypersensitivity

Quite a number of workers (e.g., Rubin and Arzichowskaja, 1948; Suchorukow, 1952; Rubin and Aksenova, 1957) tried to establish a correlation between enzymatic "equipment" and immunological behavior of the plant. Grechushnikov (1939), for instance, claimed to have found in potato varieties resistant to *Phytophthora infestans* that the activity of peroxidase is higher than in susceptible ones. In using potato varieties resistant due to hypersensitivity, Kammermann (1951) failed to find such a relation. Hassebrauk and Kaul (1957) determined the content of cytochrome oxidase, ascorbic acid oxidase, polyphenolase, and peroxidase

in wheat varieties hypersensitive to as many races of *Puccinia graminis*, *P. triticea*, and *P. glumarum* as possible. "Michigan Amber," highly susceptible to all three types of rusts, was used as a control. Moreover, plants of highly resistant varieties were treated with enzyme poisons and compared with the untreated plants for fermentation activity. The cytochrome oxidase activity was found to be smaller in eusymbiotic H/P complexes than in the parabiotic ones; the opposite was true for the behavior of ascorbic acid oxidase and polyphenolase activity toward *Puccinia triticea*. The authors considered that plants reacting in a more or less hypersensitive fashion are characterized by a "quicker metabolism" and that the content of ascorbic acid and phenols in the host plant is probably involved in determining the infection type.

### C. Hypersensitivity Reaction as "Defense Mechanism" *Sensu Stricto*

The possibility last mentioned, leads to theories according to which a defense reaction is initiated by infection which limits further growth of the pathogen in hypersensitive tissues. There are two possibilities, *a priori*: the pathogen is deprived of nutritional supplies by the above mentioned histological changes, or the activation or new buildup of one or more toxic principles is produced by the infection, and thus further penetration of the pathogen is stopped.

#### 1. Postinfection Interruption of Nutritional Supply to the Pathogen as Antipathogenic Principle?

The former possibility was considered very early (Ward, 1902a; Marryat, 1907; Stakman, 1915). It is naturally only applicable to obligate biotrophic parasites and presupposes that (1) death, or at least damage, of the host cell occurs before death of the pathogen, and (2) the pathogen releases some substances into the cell of the host that have a lethal effect on the cells of the hypersensitive host. The former supposition can be proven (e.g., Allen, 1927; Nussbaum and Keitt, 1938; Müller, 1953). Conclusive evidence for the premise mentioned second is lacking; the proof of specific toxins released by the pathogen into the cell of the host, or the *specific* reaction of the host to these, cannot be proven as yet.

Another hypothesis that also presupposes the maintenance of a living state for the establishment of a normal pathogen relationship, comes from Gassner and Hassebrauk (1938). According to this hypothesis, eusymbiotic interactions between the cell of the host and the pathogen are based on a quantitative relationship between the toxins eliminated by the pathogen into the cell of the host and the neutralizing "antitoxins" of the cell of the host. Parabiotic interactions should consequently be ascribed to the inability of the cell of the host to mobilize antitoxins



with sufficient speed. In establishing this hypothesis, Gassner and Hassebrauk relied on the experience that the sensitivity is lowered when the plant is pretreated with narcotics, whereas the nitrogen content of the tissue increases. Contrary to the concept prevailing in animal pathology, the ability of antitoxin production would be correlated with susceptibility to the disease producing agent.

## 2. Postinfection "Antibodies" as Antipathogenic Factors

A short survey of literature about HR will show that many workers have counted on the possibility that during the interaction substances are formed that have a toxic influence on the pathogen. A whole line of indications have corroborated this concept. Bernard (1909) found that embryos of *Loroglossum hircinum*, an orchid, are protected from infection with a virulent strain of *Rhizoctonia repens* by preinfection with a less "aggressive" representative of this fungus. The protection was restricted to the preinfected tissue. Similar attempts by Müller and Börger (1940) left barely any doubt that the hypersensitive cell of the host is actively involved in HR as a producer of defense factors. The way to a more dynamic interpretation of HR was paved, since during the last few years it was proven that the plant can really produce "defense bodies" in response to fungal attack (Gäumann *et al.*, 1950; Kuć *et al.*, 1955; Müller, 1956).

a. "A Priori" Indications. In obligate biotrophic pathogen (viruses included) the scientist always faces the problem as to whether the post-infection inhibition of the intruder is only caused by the premature death of the infected cell. This difficulty does not exist in the case of facultative biotrophic or necrotrophic pathogens. It can be explained herewith that important progress in causal-analytical clarification of the hypersensitivity problem was seen only after the general occurrence of HR was recognized in H/P combinations too, in which no obligate biotrophic organism participates. *Venturia* or *Colletotrichum*, can be easily cultured on many inert nutritional substrates and thus prosper better on such substrates than on the natural host. In these cases one cannot understand why the pathogen, after initial normal development in the hypersensitive tissue, should stop growth because of lack of nutrition. On the other hand, the contention that the "demarcation tissues" which often developed postinfectiously hinder further advance of the pathogen, lost its importance when it was recognized that inhibition of the pathogen can be observed even before the histological differentiation of the demarcation tissue takes place (e.g., Nobécourt, 1927). The ultimate contention that the pathogen stops growth possibly because of "self-

poisoning" (i.e., elimination of metabolites that are poisonous for the pathogen itself) cannot readily be discarded. But it is necessary, in this case too, to postulate as far as the behavior of the pathogen in a normosensitive host is concerned, that the effusion of "self-inhibitory" substances depends on the chemical composition of the tissue of the host.

b. *Experimental Indications.* It was proven with potato and *Phytophthora infestans* (Müller and Börger, 1940) that the tissue of the host is transformed during parabiotic interaction into an environment that is inhospitable to the pathogen: If the parenchymal tissue of tubers of a  $R_1$  gene carrier is inoculated with a parabiotic race of the fungus, the tissue loses its property of serving as a host tissue even for those races of the fungus that would otherwise develop a high virulence on it. Even with simultaneous inoculation of parabiotic and eusymbiotic races, the eusymbiotic partner does not develop normally. This result shows that the avirulent, not the virulent, strain determines the "condition" into which the host tissue is transformed during interaction. Since both races can grow normally one next to the other in a narrow space in tubers that are susceptible to them both, this "inhospitable" condition—that is brought on in the resistant tuber by preloading with a parabiotic *Phytophthora* race—cannot be based on an antagonism between the two races. Thus, this must be necessarily due to an interaction in which the host is equally involved with the pathogen that interacts with it parabolically.

The necrotic tissue does not accept other organisms that grow on living potato tubers (e.g., *Fusarium caeruleum*) or on tuber slices killed by heat. Thus, lack of nutritional substances cannot be considered the cause for inhibition of fungal growth in that parabolically reacting tissue. But no total immunization of the tuber takes place. The eusymbiotic race can develop normally outside of the "vaccinated" tissue area and its immediate surrounding, directly attacked by the parabiotic race.

From the foregoing and the fact that there is a close correlation between reaction rate and resistance degree, we may conclude that a principle is activated or newly formed in the parabolically interacting tissues that has a toxic effect on the microorganisms. This principle could be an "antibody" in the original sense of the word. But, since the animal pathologist associates the concepts of specificity and humoral immunity with this term, the postulated antibiotic principle is called "phytoalexin." This term refers only to the function as "antibody"; it does not include any statement about the chemical structure of the active principle.

Arnaudi (1942), studying a problem similar to the one of Müller

and Börger, soon afterward established that in the same H/P combination, injection of potato tubers with parabiatic races of the causative agent gives a local protection against attack by eusymbiotic races.

Johnston's and Huffman's (1958) experiments explored the existence of an antagonistic effect of rust fungi *in vivo*. Their study points in the same direction: if wheat leaves are covered with spores of *Puccinia coronata*, a pathogen of oats, the germ tubes of the fungus penetrate into the host tissue but only elicit "heavy flecking" there. If the leaves are infected two days later with a virulent race of *Puccinia triticina*, the frequency of microscopically recognizable *triticina* infections is strongly reduced; on the other hand, only necrosis occurs in the areas preinfected with *P. coronata* (reaction type 1). Outside of the preinoculated area, reaction is normal (reaction type 4). The writers leave open the question whether an effect of substances "produced by the latent mycelium of an organism (i.e., *Puccinia coronata*) that was not able to establish itself parasitically," is involved or only a purely mechanical blocking of the stomata through preinfection. Neither might occur, according to the results obtained with experiments on potatoes. Most probably the inhibition of *Puccinia triticina* in the mesophyll is due to changes in chemical constituents after infection with the parabiatic partner.

c. *Demonstration of Interaction Products with Antibiotic Effect.* Demonstration of such substances succeeded under adequate experimental conditions in combinations, *Phaseolus vulgaris* (inner epidermis of seed cavities) and *Phytophthora infestans*, and *Ph. vulgaris* and *Sclerotinia fructicola*, that interact "in a hypersensitive fashion" (Müller, 1956; see also pp. 477-478). In three other hosts (*Capsicum annuum*, *Pisum sativum*, and *Vicia faba*), the same result was achieved after infection with the above mentioned pathogens (Müller, 1958a). It was also shown that development of the antibiotic principle occurs a few hours after infection in parabiatically reacting tissues. Under the experimental conditions used, the effective principle is developed and diffuses from the site of interaction in quantities a thousand times greater than necessary for preventing fungal growth at the focus of infection.

The specificity of the effective principle (phytoalexin) is small. For instance, it is effective against *Uromyces trifolii* or *Colletotrichum lindemuthianum*. It passes through semipermeable membranes (e.g., Cellophane) which points to a relatively small molecular weight; it has properties that characterize it as a "hydrophilic lipid." It is effective within a pH range of 4.0 to 7.5. Its antibiotic effectiveness is not influenced by the presence of substances that could serve *in vivo* as nutritional substances for the pathogen. The output capacity increases with increasing age of the host tissue. This recalls the fact that older plants

are usually more sensitive than young plants ("age resistance"). The phytoalexin is mostly adsorbed by the parasitized cells and surrounding tissue. This might be especially conclusive for the understanding of the HR mechanism. Because, if the phytoalexin is not fixed, larger and larger quantities would flow into the surrounding tissue, and the concentration of phytoalexin critical for the pathogen would be attained much later or not at all at the focus of infection.

As mentioned previously (see page 491), short-term treatment of tissue of the host with high, but not lethal, temperatures leads to a temporary blocking of HR. As expected, it was found that the temporarily "desensitized" tissues are incapable of responding to the infection with production of phytoalexin. When, however, the host tissue regains its original sensitivity, phytoalexin elimination also becomes normal (Jerome and Müller, 1958).

The question is whether those results can be applied to H/P combinations when an obligate biotrophic parasite is involved. Even though much seems in favor of this concept, a generalization of findings obtained by Müller (1956, 1958a) in "unnatural" H/P combinations is not yet permissible. In the case of obligate parasitism, one should take into account the possibility that specific nutritional or growth substances, essential for the life of the parasite, may be of greater significance for the initiation of HR.

## VI. PROBLEM OF "ACQUIRED IMMUNITY"

The concept of "acquired immunity" is, like that of hypersensitivity, borrowed from human pathology. It is based on the experience that a host once cured of disease has a more or less prolonged defensive protection against a second infection. Contrary to this "acquired" immunity, is considered "inherent" ("natural" or "congenital") immunity, characterized by the fact that it is inherent in the organism from the beginning of its development. When it was proven in animals at the turn of this century, that the "condition" of acquired immunity is associated with the existence of specific circulating "antibodies" in the blood, attempts were made to prove a similar defense mechanism in plants. Based on experiences in mammals, attempts were made to reach that goal in two ways. First, to demonstrate that the resistance of the plant is increased by preinfection with "attenuated" strains or by "vaccination" with metabolic products of the homologous causative agent. Second, lysin-, precipitin-, or agglutinin-like bodies that protect the plant from attack of a potential parasite were searched for. Of secondary significance was the question whether preformed or postinfectiously obtained specific "antibodies," are involved as in animal pathology.



In the following attempt to summarize the result of these efforts, all resistance changes should be excluded that can be obtained by systemic application of chemotherapeutics, e.g., sulfonamides or antibiotics, or by manipulation of external circumstances. By definition they do not belong into the category of "acquired" immunity. On the other hand, this concept should not be taken too literally. Thus, it would not be right to exclude from the following considerations all cases in which immunization of the whole plant fails to be evident. This is even less indicated because research in animal pathology only recently began to pay more attention to the phenomenon of locally acquired immunity and to material changes of the host tissue that are limited to the infection focus. Also, the existence of acquired immunity should not be linked with the postinfection appearance of specific "antibodies." In animals, they only make the pathogen more susceptible toward the resistance mechanism linked to the diseased tissue. The interpretation of animal pathologists predominantly tends toward the conclusion that the tissue defense reactions of the immune and non-immunized animal are qualitatively similar and that after acquisition of the immune state the existing reactions appear more rapidly and heightened with greater intensity. No new reactions appear (Miles and Wilson, 1950).

No survey of the subject acquired immunity in plants can be given within the framework of this article. Chester's (1933) and Vavilow's (1935) summary of the problem and the literature cited by Gäumann (1951) and Hess (1949) provide a basic bibliography. A few strategically important papers are reported as follows.

The first attempts to prove the existence of "acquired immunity" within the plant kingdom stem from the French scientists Beauverie (1901) and Ray (1901). They reported success in obtaining an increased resistance in their experimental plants through inoculation of the soil with, or by direct application of, avirulent strains of the disease-producing agents in question.\* However, the interpretation of these experimental findings as a proposed working hypothesis was accepted with considerable skepticism by many phytopathologists. This was due, on the one hand, to lack of precision of the published results, and on the other to the fact that the results of both authors as well as of later authors could not be corroborated. Finally, it was simply denied, from purely theoretical considerations that the plant has the ability to "acquire" resistance. The main arguments were lack of a circulation system corresponding to blood circulation of animals and the unsuccessful attempts of other scientists to prove the presence of antibodies in

\* Other "vaccines" too, e.g., killed cultures or mycelial extracts, were used by these and later authors.

resistant or immunized plants that could be compared to the classic antibodies of animals (Silberschmidt, 1932; Carbone, 1936).

If we exclude from our observations all studies that do not have unequivocal results, or whose results could not be confirmed by later authors, the general interpretation points to the fact that plants cannot respond to infection with constitutional changes that protect the whole body against a second infection. Although it was established that a reduction of attack rate or severity of disease can be effected by pre-treatment of germinating seeds or young plants with culture filtrates or other "vaccines" (e.g., Nobécourt, 1927; Carbone, 1934), it was mostly not ascertained whether the immunization effect was caused by changes in the reaction potential or purely by a decrease in infection rate per unit of plant surface exposed to the pathogen. The results obtained by Zoja (1925) and Hess (1949) in barley or wheat and *Helminthosporium sativum* are very conclusive: both authors were able to achieve a clear reduction of disease attack in young plants that have been transplanted into the open after the seeds were cultured on a substrate to which ground mycelium of the disease-producing agent was added as a "vaccine." However, Hess showed that the young plants suffer greatly under vaccination (poor development of roots, slower growth in comparison to controls); also, the same immunological effect could be achieved when young plants are cultured in highly concentrated nutritional solutions to which no ground *Helminthosporium* mycelium was added. Hess, to whom we are indebted for the most critical studies of this problem leaves the question open whether the "vaccination results" obtained by him and Zoja can be actually considered an immunization of plants in the true sense of the word. They could also be the result of an unspecific displacement of the reaction potential that corresponds to what we can, for instance, observe after treatment of plants with antibiotics, etc.

Where resistance is increased locally, the situation is clearer. As previously mentioned, the attacked tissue becomes more inhospitable for the parasite in parabiotic H/P complexes. The range of inhospitality produced by the primary infection is most significant. However, Bernard's (1909) studies in *Loroglossum* seedlings and *Rhizoctonia repens* and *Rh. lanuginosa*, as well as the studies of Müller and Börger (1940) in potato tubers and *Phytophthora infestans* showed that the range does not exceed a relatively limited number of cell layers around the necrotic tissue. On the other hand, Gäumann *et al.* (1950) demonstrated the antibiotically effective substance, produced by *Rhizoctonia repens* metabolites in orchid bulbs, even at a distance of 20 mm. from the tissue complex, that had been under direct influence of the parasite's metabolites. This points to the fact that some secretion product of *Rh. repens*

diffuses further into the tissue of the host and induces the formation of the antibiotically effective substance.

The *Pyrus communis* and *Viscum album* case should be mentioned as an outstanding example of "sensitization at a distance" (Heinricher, 1929, Paine, 1950, Gäumann, 1956). In the pear species "William Christ," that is characterized by an inherent lability, sensitivity of the trees increases under consecutive yearly infections with mistletoe; this means that the tendency to react to new infections with necrosis and to cut off the penetrating mistletoe seedlings from the remaining tissue rises. But this sensitization goes no further than the infected branch of the tree. Thus, here too, in this extreme case, the host cannot acquire immunity as a whole.

This evident inability of the plant to acquire "humoral" immunity, prompted many authors to feel that immunological effectiveness of the plant is limited to the "inherent" immunity type. This thesis, however, can be applied only to cases where freedom from attack would depend on the presence of preformed resistance factors. In the case of resistance due to hypersensitivity the thesis misses the point. In this case immunity itself is basically not "inherent." Only the reaction potential is inherent. This enables the plant to localize the disease-producing agent at the infection focus. In other words: only the ability to achieve resistance at the infection site, and at this site alone, is inherited, not the resistant state itself. The state of resistance is acquired only when the plant comes in contact with the causative agent. Then the mechanism becomes operative which changes the tissue parts, attacked by the parasite, from an "indifferent" into a "resistant" state.

## VII. EPILOGUE

An attempt was made above to survey the present status of our knowledge on the essence and mechanism of HR. Such an attempt necessarily must leave a feeling of dissatisfaction behind it. This is true because the accumulated experiences are not sufficient to correlate them logically with an acceptable theory of hypersensitivity. But our knowledge is sufficient to present a hypothetical picture that brings us closer to an understanding of what we consider to be a hypersensitivity reaction. Let us proceed from the following arguments:

1. HR is a process in which the pathogen is just as actively involved as the host.
2. As in every living process, the course and clinical result of HR is controlled by a whole series of various factors.
3. As already recognized by Ward, the interaction ending in the

death of both partners cannot be explained on a completely nutritional-physiological basis.

4. On the other hand, it has been possible to show, under greatly simplified experimental conditions, that a substance toxic for the pathogen is eliminated after infection.

5. The formation of this substance is correlated, in space and time, with the physiological breakdown of the cell of the host *as well as* of the pathogen.

6. The HR system can be inactivated temporarily by manipulation of external circumstances; simultaneously, the tissue loses its ability to respond to infection with production of an antipathogenic substance.

These findings do not leave any doubt that the postinfection formation of such a substance, at least in cases studied up to now, is the direct reason for the antipathogenic character of HR. Thus, HR can be considered a defense reaction *sensu stricto*.

Whether we call this defensive principle antibody or something else, is a question of terminology. The writer prefers the term phytoalexin, so that confusion with the classic antibodies of animals is avoided. Such a terminological demarcation is even more strongly indicated since phytoalexin is a defense principle directly aimed against the pathogen.

What then is the nature of the system that we can hold responsible for the postinfection formation of phytoalexins? We have to rely here mainly on suppositions. But much evidence points to the fact that HR is an enzymatic interaction in which reactants are involved that are supplied by the cell of the host as well as by the pathogen. The fact that the mechanism underlying HR is very sensitive to narcotics and enzyme inhibitors is a strong argument in favor of this concept. Also genetic arguments favor such a concept. According to the present concept, the genes—represented by their dominant alleles—have an enzyme-producing function. Our hypothesis agrees with this idea because the genes that control resistance of the host or avirulence of the pathogen have been proven mostly to be dominant. Consequently, the gene-for-gene system drafted by Flor (1955) and others and expressed in physiological terms represents a reactant-for-reactant system.

At first, this conclusion seems to disagree with the fact that antibiotic effectiveness of the heretofore proven phytoalexins is nonspecific. This only appears to be a dilemma. Actually, the specific alternative is not hypersensitivity, but normosensitivity. This is seen clearly when we transfer parasites such as *Phytophthora infestans* into "wrong host plants" (e.g., Müller, 1950). Then it is found that "hypersensitivity" not "normosensitivity" represents the norm. The fact that normosensitive behavior is considered the rule is purely because we recognize the existence of a



pathogen only when it goes astray onto a normosensitive host. The unsuccessful attempts of the pathogen to take hold on a hypersensitive host plant escape our notice under natural conditions.

Resistance due to hypersensitivity was explained differently as the result of a phylogenetic adjustment process of the host plant to its surrounding parasitic flora. This thought presupposes that the respective plant type or its phylogenetic ancestors were in direct contact with the potential disease-producing agent for a longer period of time. Upon closer examination, such a theory proves faulty, at least within this context. Thus, plants that can be found naturally only in the arid climate areas of Australia (e.g., *Erythrina vespertilia* Benth., *Clianthus formosus* Ford et Vick. and *Cassia sturtii* R.Br.), proved to be hypersensitive to *Phytophthora infestans*, a pathogen restricted to humid climates. In this case, the ability to respond to the attack of the pathogen with HR, cannot possibly be the result of an "adaptation process." Another important argument: the center of *Phytophthora infestans* should be searched for, according to the latest studies, in the southern part of the North American continent. Hypersensitivity should thus undoubtedly be considered a phylogenetically original situation. In the process of mutual adjustment, above all only the parasite is the active (i.e., through mutation and recombination), the adapting partner; the host has only the passive role of a selection sieve. On the other hand, once the pathogen has taken hold on the new, normosensitive, host, a second selective process counteracting the first one begins in which the hypersensitivity of the host plant represents the positive selection mark. Of course, as unfortunately the experiences of plant breeders have shown, in the long run the pathogen is always the superior partner because of its greater multiplying frequency as compared to that of the host.

The mechanism underlying HR deserves the interest of the biologists even beyond the purely pathological. Why, we may ask, are so many microorganisms incapable of utilizing and destroying "tender" tissue, such as that of a carrot root, even though they thrive richly on all sorts of artificial nutritional media and are characterized by low susceptibility to toxic substances of a higher plant? This question seems trivial, since we are accustomed to calling such organisms saprophytes in contrast to the "true" parasites. But the question appears in a different light when we think of the fact that according to the simple nutrition-inhibition concept such an organism should be more virulent than a pathogen which can be cultivated only on special nutritional media or even only on a living host. To explain this situation by attributing the pathogenic inability of a saprophyte to the "natural" resistance of a living host tissue would be the same as an attempt to explain daylight by saying the sun

is shining. Such a statement makes sense only when we can say what the resistance of the living host tissue is based upon. We have seen that the number of microorganisms which can penetrate into the tissue of a given host plant without producing the above described HR is negligibly small in comparison with the number of potential pathogens. This fact suggests that a pathogenic relationship can be established only when HR is not released by the infection. We should postulate such a mechanism for each normal living tissue. Noll's (1949) studies on the behavior of wheat leaves toward *Penicillium glaucum*, a common saprophyte indicate that this thought might not be too misleading—namely, when the wheat leaves are exposed to a temperature of 50° C. for 30 to 50 seconds, the fungus can penetrate into the leaf tissue and there elicit a local decay. After a certain period of time, the leaf regains its resistance, i.e., the fungus stops its growth again. But, at the same time histological changes occur in the immediate vicinity of the tissue areas occupied by the fungus, which have much in common with those that are observed after a leaf is infected with a parabiotic rust race. Isn't perhaps the defense mechanism functioning here that we have postulated as the classic hypersensitivity reaction? This question has not been answered as yet with certainty. Only more experimental work can give an answer to it.

## REFERENCES

- Allen, P. J., and D. R. Goddard. 1938. Respiratory study of powdery mildew of wheat. *Am. J. Botany* **25**: 613–621.
- Allen, R. F. 1923. A cytological study of infection of Baart and Kanred wheats by *Puccinia graminis tritici*. *J. Agr. Research* **23**: 131–151.
- Allen, R. F. 1927. A cytological study of orange leaf rust, *Puccinia triticina* physiologic form 11, on Malakoff wheat. *J. Agr. Research* **34**: 697–714.
- Angell, H. R., J. C. Walker, and K. P. Link. 1930. The relation of protocatechuic acid to disease resistance in the onion. *Phytopathology* **20**: 431–438.
- Arnaudi, C. 1942. Recenti acquisizioni in tema d'immunità vegetale. *Riv. patol. vegetale* **5**: 1–20. From Hess (1949).
- Bandlow, G. 1951. Mutationsversuche an Kulturpflanzen. II. Züchterisch wertvolle Mutanten bei Sommer- und Wintergersten. *Züchter* **21**: 357–363.
- Beauverie, J. 1901. Essais de l'immunisation des végétaux contre les maladies cryptogamiques. *Compt. rend.* **133**: 107–110.
- Behr, L. 1949. Über den Einfluss von narkotisch wirkenden Stoffen auf die Wundperidermbildung und die Resistenz der Kartoffelknolle gegenüber *Phytophthora infestans* und Vertretern der Gattung *Fusarium*. *Phytopathol. Z.* **15**: 407–446.
- Bennet, C. W. 1953. Interactions between viruses and virus strains. *Advances in Virus Research* **1**: 39–67.
- Bernard, N. 1909. Remarques sur l'immunité chez les plantes. *Bull. inst. Pasteur* **7**: 369. From Hess (1949).
- Bever, W. M. 1934. Effect of light on the development of uredial stage *Puccinia glumarum*. *Phytopathology* **24**: 507–516.

- Biffen, R. H. 1907. Studies in the inheritance of disease resistance. *J. Agr. Sci.* **2**: 109-128.
- Börner, C., and F. A. Schilder. 1932. Aphidoidea, in Sorauer's Handbuch Pflanzenkrankh. **5**(2): 703-705.
- Börner, C., and F. A. Schilder. 1934. Beiträge zur Züchtung reblaus- und mehltaufester Reben. *Mitt. Biol. Zentralanstalt (Reichsanstalt.) Land-u. Forstwirtschaft* **49**.
- Brown, W. 1955. On the physiology of parasitism in plants. *Ann. Appl. Biol.* **43**: 325-341.
- Browning, J. A. 1954. Breakdown of rust resistance in detached leaves of normally resistant oat varieties. *Phytopathology* **44**: 483 (Abstr.)
- Carbone, D. 1934. L'immunità vaccinale nelle piante. *Natura* **25**: 111-134.
- Carbone, D. 1936. Le reazione umorali delle piante. *Rappt. III ième Congr. Intern. Pathol. Comp. (Athènes)* **1** (2nd part): 65-72.
- Carbone, D., and A. V. Alexandri. 1935. Recherches sur les anticorps chez les végétaux. *Boll. Sez. ital. soc. intern. microbiol.* **6**: 1-5.
- Catcheside, D. G. 1949. "The Genetics of Micro-organisms." Pitman, London.
- Chessin, M., and H. A. Scott. 1955. Mineral nutrition and the size of local lesions induced by TMV. *Science* **121**: 112.
- Chester, K. S. 1933. The problem of acquired physiological immunity in plants. *Quart. Rev. Biol.* **8**: 129-154, 275-324.
- Chitwood, B. G., and B. A. Oteifa. 1952. Nematodes parasitic to plants. *Ann. Rev. Microbiol.* **6**: 151-184.
- Christiansen-Weniger, E. 1955. Versuche zur stoffwechselphysiologischen Beeinflussung der Reaktion der Kartoffelknolle auf *Phytophthora infestans* de By. *Phytopathol. Z.* **25**: 153-180.
- Comes, O. 1913. Della resistenza dei frumenti alle ruggini. *Atti Ist. incoraggiamento sci. nat. Napoli*. From Zimmermann (1925).
- Comer, E. J. H. 1935. Observations on resistance to powdery mildews. *New Phytologist* **34**: 180-200.
- Cruickshank, I. A. M., and T. Swain. 1956. Study of phenolic compounds in oil-flax. *J. Exptl. Botany* **7**: 410-415.
- De Bruyn, H. L. 1943. Method voor het vaststellen van de vatbaarheids-grad van aardappel knollen voor de aardappelziekte. *Tijdschr. Plantenziekten* **49**: 77-99.
- Doak, K. D. 1930. Necrotic effect produced by wilting of susceptible varieties of wheat infected with leaf rust. *Phytopathology* **20**: 120-121.
- Dufrenoy, J. 1936. Rôle des amino-acides et des composés phenoliques dans la susceptibilité ou la résistance des plantes aux maladies. *Rappt. III ième Congr. Intern. Pathol. Comp. (Athènes)* **1** (2nd part): 16-38.
- Dufrenoy, J., and M. L. Dufrenoy. 1934. Cytology of plant tissues affected by viruses. *Phytopathology* **24**: 599-619.
- Eide, C. J. 1955. Fungus infection in plants. *Ann. Rev. Microbiol.* **9**: 297-318.
- Esau, E. 1938. Some anatomical aspects of plant virus disease problems. *Botan. Rev.* **4**: 548-579.
- Farkas, C. L., and Z. Király. 1958. Enzymological aspects of plant diseases. I. Oxidative Enzymes. *Phytopathol. Z.* **31**: 251-272.
- Ferris, V. R. 1955. Histological study of pathogen-suscept relationships between *Phytophthora* infections and derivatives of *Solanum demissum*. *Phytopathology* **45**: 546-552.
- Fischer, E., and E. Gäumann. 1929. "Biologie der pflanzenbewohnenden parasitischen Pilze." G. Fischer, Jena.

- Flentje, N. T. 1957. Studies on *Pellicularia filamentosa* (Pat.) Rogers III. Host penetration and resistance, and strain specialization. *Brit. Mycol. Soc. Trans.* **40**: 322-336.
- Flor, H. H. 1955. Host-parasite interaction in flax-rust. Its genetics and other implications. *Phytopathology* **45**: 680-685.
- Freisleben, R., and A. Lein. 1942. Über die Auffindung einer mehltau-resistenten Mutante nach Röntgen-Bestrahlung einer anfälligen reinen Linie von Sommergerste. *Naturwissenschaften* **30**: 608.
- Frey, K. J., and J. A. Browning. 1955. Mutations for stem rust resistance induced in oats by X-ray treatment. *Phytopathology* **45**: 490-492.
- Fuchs, A., J. Grosjean, J. M. Krythe, and T. W. Reijenga. 1957. Bacteriekanker bij Steenvruchten. I. Symptomen en ziekteverloop bij kers en pruim. *Tijdschr. Plantenziekten*. **63**: 33-44.
- Fuchs, W. H., and E. Kotte. 1954. Zur Kenntnis der Resistenz von *Solanum tuberosum* gegen *Phytophthora infestans*. *Naturwissenschaften* **41**: 169-170.
- Futrell, M. C., and J. G. Dickson. 1954. The influence of temperature on the development of powdery mildew on spring wheats. *Phytopathology* **44**: 247-251.
- Garber, E. D. 1956. A nutrition-inhibition hypothesis of pathogenicity. *Am. Naturalist* **40**: 183-194.
- Gassner, G., and W. Franke. 1934. Der Stickstoffhaushalt junger Weizenpflanzen in seiner Abhängigkeit von der Mineralsalznährung. Ein Beitrag zum Problem der Rostresistenz. *Phytopathol. Z.* **7**: 187-222.
- Gassner, G., and G. Goetze. 1936. Einige Versuche über die physiologische Leistungsfähigkeit rostinfizierter Getreideblätter. *Phytopathol. Z.* **9**: 371-386.
- Gassner, G., and K. Hassebrauk. 1931. Untersuchungen über die Beziehungen zwischen Mineralsalznährung und Verhalten der Getreidepflanzen gegen Rost. *Phytopathol. Z.* **3**: 535-617.
- Gassner, G., and K. Hassebrauk. 1933. Über die Beeinflussung der Rostanfälligkeit durch Eintauchen geimpfter Blätter in Lösungen von Mineralsalzen und anderen Stoffen. *Phytopathol. Z.* **5**: 323-342.
- Gassner, G., and K. Hassebrauk. 1936. Untersuchungen zur Frage der Getreiderostbekämpfung mit chemischen Mitteln. *Phytopathol. Z.* **9**: 427-454.
- Gassner, G., and K. Hassebrauk. 1938. Untersuchungen über den Einfluss von Äther- und Chloroformnarkose auf das Rostverhalten junger Getreidepflanzen. *Phytopathol. Z.* **11**: 47-97.
- Gassner, G., and W. Straib. 1930a. Untersuchungen über die Abhängigkeit des Infektionsverhaltens der Getreiderostpilze vom Kohlensäuregehalt der Luft. *Phytopathol. Z.* **1**: 1-30.
- Gassner, G., and W. Straib. 1930b. Experimentelle Untersuchungen über das Verhalten der Weizensorten gegen *Puccinia glumarum*. *Phytopathol. Z.* **1**: 215-255.
- Gäumann, E. 1948. "Pflanzliche Infektionslehre." Birkhäuser, Basel.
- Gäumann, E. 1951. "Pflanzliche Infektionslehre," 2nd ed. Birkhäuser, Basel.
- Gäumann, E. 1952. Über Abwehrreaktion bei Pflanzen. *Zentr. Bakteriол. Parasitenk. Abt. II.* **158**: 205-217.
- Gäumann, E. 1956. Über Abwehrreaktionen bei Pflanzenkrankheiten. *Experientia* **12**: 411-418.
- Gäumann, E., R. Braun, and G. Bazzigher. 1950. Über induzierte Abwehrreaktionen bei Orchideen. *Phytopathol. Z.* **17**: 36-62.
- Gradinaroff, L. 1943. Über die Ätiologie komplexbedingter Knollenfäulen bei der Kartoffel. *Arb. Biol. Reichsanstalt. Land-u. Forstwirtschaft, Berlin-Dahlem* **23**: 405-428.



- Grechushnikov, A. J. 1939. Role of peroxidase in immunity against *Phytophthora infestans* de Bary (russ.). *Compt. rend. Acad. Sci. U.R.S.S.* **25**(8). (From Kattermann, 1951).
- Green, G. J., and J. G. Dickson. 1957. Pathological histology and varietal reactions in *Septoria* leaf blotch of barley. *Phytopathology* **47**: 73-79.
- Greenham, C. G., and K. O. Müller. 1956. Conductance changes and responses in potato tubers following infection with various strains of *Phytophthora infestans* and with *Pythium*. *Australian J. Biol. Sci.* **9**: 199-212.
- Harrison, B. D. 1956. The infectivity of extracts made from leaves at intervals after inoculation with viruses. *J. Microbiol.* **15**: 210-220.
- Hart, H. 1926. Factors affecting the development of flax rust, *Melampsora lini* (Pers.) Lev. *Phytopathology* **16**: 185-205.
- Hassebrauk, K. 1940. Zur Frage des Einflusses der Aussenfaktoren auf verschiedene Stadien von Weizenbraunrostinfektionen. *Phytopathol. Z.* **12**: 490-510.
- Hassebrauk, K. 1951. Untersuchungen über die Einwirkung von Sulfonamiden und Sulfonen auf Getreideroste. I. Beeinflussung des Fruktifikationsvermögens. *Phytopathol. Z.* **17**: 384-400.
- Hassebrauk, K., and R. Kaul. 1957. Vergleichende chemische Untersuchungen des Atmungsstoffwechsels von Weizenpflanzen unterschiedlicher Braunrostanfälligkeit. *Phytopathol. Z.* **29**: 305-326.
- Hayden, E. B. 1956. Pathogenicity of races 11, 15B, 49, 125 and 139 of *Puccinia graminis* var. *tritici* to new spring wheats. *Phytopathology* **46**: 145-150.
- Heinricher, E. 1929. Allmähliches Immunwerden gegen Mistelbefall. *Planta* **7**: 165-173.
- Hess, H. 1949. Ein Beitrag zum Problem der induzierten Abwehrreaktionen im Pflanzenreich. *Phytopathol. Z.* **16**: 4-70.
- Hirata, K. 1956. Some observations on the relation between penetration hypha and haustorium of the barley mildew (*Erysiphe graminis*) and the host cell. II. On the collapse of mesophyll cells of the barley leaves attacked by the mildew. *Ann. Phytopathol. Soc. Japan* **21**: 23-28.
- Holmes, F. O. 1929. Local lesions in tobacco mosaic. *Botan. Gaz.* **87**: 39-55.
- Holmes, F. O. 1932. Symptoms of tobacco mosaic disease. *Contribs. Boyce Thompson Inst.* **4**: 323-357.
- Holmes, F. O. 1934. Inheritance of ability to localize tobacco-mosaic virus. *Phytopathology* **24**: 984-1002.
- Holmes, F. O. 1937. Inheritance of resistance to tobacco-mosaic disease in the pepper. *Phytopathology* **27**: 637-642.
- Honecker, L. 1934. Über die Modifizierbarkeit des Befalles und das Auftreten verschiedener physiologischer Formen beim Mehltau der Gerste, *Erysiphe graminis hordei*. *Z. Züchtungsbil.* **A19**: 577-602.
- Hooker, A. L. 1956. Correlation of resistance to eight *Pythium* species in seedling corn. *Phytopathology* **46**: 175-176.
- Hori, M. 1935. Studies on the relation of *Phytophthora infestans* (Mont.) De Bary to resistant plants. *Ann. Phytopathol. Soc. Japan* **5**: 225-244.
- Hotson, H. H. 1953. Some chemotherapeutic agents for wheat stem rust. *Phytopathology* **43**: 659-662.
- Humphrey, H. B., and J. Dufrenoy. 1944. Host parasite relationship between oat plant (*Avena* spp.) and crown rust (*Puccinia coronata*). *Phytopathology* **34**: 21-40.
- Hurd, A. M. 1924. The course of acidity changes during the growth period of wheat with special reference to stem rust resistance. *J. Agr. Research* **27**: 725-735.

- Hursh, C. R. 1924. Morphological and physiological studies on the resistance of wheat to *Puccinia graminis tritici* Erikss. et Henn. *J. Agr. Research* **27**: 381-411.
- Husfeld, B. 1931. Über die Züchtung plasmoparawiderstandsfähiger Reben. *Gartenbauwissenschaft* **7**: 15-92.
- Jennings, P. R., and A. J. Ullstrup. 1957. A histological study of three *Helminthosporium* leaf blights in corn. *Phytopathology* **47**: 707-714.
- Jerome, S. M. R., and K. O. Müller. 1958. Studies in Phytoalexins. II. Influence of temperature on resistance of *Phaseolus vulgaris* towards *Sclerotinia fructicola* (Wint.) Rehm, with reference to phytoalexin output. *Australian J. Biol. Sci.* **11**: 301-314.
- Johnson, T. 1931. A study of the effect of environmental factors on the variability of physiologic forms of *Puccinia graminis tritici* Erikss. et Henn. *Can. Dept. Agr. Bull.* **140**.
- Johnson, T. 1953. The rust of cereals. *Biol. Revs. Cambridge Phil. Soc.* **28**: 105-157.
- Johnston, C. O., and M. D. Huffman. 1958. Evidence of local antagonism between cereal rust fungi. *Phytopathology* **48**: 69-70.
- Kammermann, N. 1951. Undersökningar rörande potatisbladmöglet *Phytophthora infestans* (Mont.) De By. II. Sambandet mellan potatisbladsaftens peroxidasaktivitet och Phytophthoraresistenten. *Statens Växtskyddsanstalt, Medd.* **58**: 1-32.
- Kargopolova, N. N. 1937. Immunität der landwirtschaftlichen Pflanzen gegenüber Krankheiten und Schädlingen (russ.). Cited by Rubin and Arzichowskaja (1953) and *Rev. Appl. Mycol.* **16**: 23.
- Keitt, G. W., and D. M. Boone. 1954. Induction and inheritance of mutant characters in *Venturia inaequalis* in relation to its pathogenicity. *Phytopathology* **44**: 362-370.
- Király, Z., and G. L. Farkas. 1955. Über parasitogen induzierte Atmungssteigerung beim Weizen. *Naturwissenschaften* **42**: 213-214.
- Király, Z., and G. L. Farkas. 1957. Decrease in glycolic acid oxidase activity of wheat leaves infected with *Puccinia graminis* var. *tritici*. *Phytopathology* **47**: 277-278.
- Király, Z., and J. Lelley. 1956. Contributions to the hypersensitive reaction of wheat to loose smut (*Ustilago tritici* [Pers.] Rostr.) infection. *Phytopathol. Z.* **26**: 143-146.
- Klimke, A. 1941. Untersuchungen über die Corynespora-Krankheit der Gurken und die Resistenz deutscher Gurkensorten. *Phytopathol. Z.* **13**: 401-435.
- Köhler, E. 1928. Fortgeführte Untersuchungen über den Kartoffelkrebs (II, III). *Arb. Biol. Reichsanstalt Land-u. Forstwirtsch. Berlin-Dahlem* **15**: 135-176, 401-416.
- Köhler, E. 1931. Über das Verhalten von *Synchytrium endobioticum* auf anfälligen und widerstandsfähigen Kartoffelsorten. *Arb. Biol. Reichsanstalt Land-u. Forstwirtsch. Berlin-Dahlem* **19**: 263-284.
- Köhler, E. 1951. Über die Bildung nekrotischer Zonen an virusinfizierten Tabakblättern. Zugleich ein Beitrag zur Frage der Virusbewegung im Blattparenchym. *Phytopathol. Z.* **17**: 115-127.
- Kuč, J. 1957. A biochemical study of the resistance of potato tuber tissue to attack by various fungi. *Phytopathology* **47**: 676-680.
- Kuč, J., A. J. Ullstrup, and F. W. Quakenbusch. 1955. Production of fungistatic substances by plant tissue after inoculation. *Science* **122**: 1186-1187.

- Leach, J. G. 1919. The parasitism of *Puccinia graminis tritici* Erikss. et Henn. and *Puccinia graminis tritici compacti* Stak. et Piem. *Phytopathology* **9**: 59–88.
- Leach, J. G. 1923. The parasitism of *Colletotrichum lindemuthianum*. Minn. Agr. Expt. Sta., Tech. Bull. **14**.
- Lehman, S. G. 1958. Physiologic races of the downy mildew fungus on soya beans in North Carolina. *Phytopathology* **48**: 83–86.
- Lepik, E. 1930. Untersuchungen über den Biochemismus von Knollenfäulen. *Phytopathol. Z.* **1**: 49–109.
- Lewis, R. W. 1957. A graphic presentation of the balance hypothesis of parasitism. *Acta Botanica Acad. Sci. Hung.* **3**: 27–29.
- Livingston, J. E. 1953. The control of leaf and stem rust with chemotherapeutants. *Phytopathology* **43**: 496–499.
- Loegering, W. Q., and J. R. Geis. 1957. Independence in the action of three genes conditioning stem rust resistance in red Egyptian wheat. *Phytopathology* **47**: 740–741.
- Marryat, D. 1907. Notes on the infection and histology of two wheats immune to the attacks of *Puccinia glumarum*, yellow rust. *J. Agr. Sci.* **2**: 129–138.
- Meyer, G. 1940. Zellphysiologische und anatomische Untersuchungen über die Reaktion der Kartoffelknolle auf den Angriff der *Phytophthora infestans* bei Sorten verschiedener Resistenz. *Arb. biol. Reichsanstalt Land- u. Forstwirtsch., Berlin-Dahlem* **23**: 97–132.
- Meyer, H. 1951. Über den Einfluss von Cadmium auf die Krankheitsbereitschaft des Weizens für *Erysiphe graminis tritici* Marchal. *Phytopathol. Z.* **17**: 63–80.
- Miles, A. A., and G. S. Wilson. 1950. Immunity and immunization. In "Chamber's Encyclopaedia." London.
- Millerd, A., and K. Scott. 1955. A phytopathogenic toxin formed in barley infected with powdery mildew. *Australian J. Sci.* **18**: 63–64.
- Millerd, A., and K. Scott. 1956. Hostpathogen relations in powdery mildew of barley. *Australian J. Biol. Sci.* **9**: 37–44.
- Minkevicius, A. 1932. Untersuchungen über den Einfluss der Narkose auf die Pilzempfindlichkeit der Pflanzen. *Phytopathol. Z.* **5**: 99–152.
- Müller, K. O. 1930. Über die Phytophthoraresistenz der Kartoffel und ihre Vererbung. *Angew. Botan.* **12**: 299–324.
- Müller, K. O. 1931. Über die Entwicklung von *Phytophthora infestans* auf anfälligen und widerstandsfähigen Kartoffelsorten. *Arb. biol. Reichsanstalt. Land- u. Forstwirtsch. Berlin-Dahlem* **18**: 465–505.
- Müller, K. O. 1935. Über den augenblicklichen Stand unserer Kenntnisse zur biologischen Spezialisierung des Krautfäuleerregers der Kartoffel (*Phytophthora infestans*). *Züchter* **7**: 5–12.
- Müller, K. O. 1950. Affinity and reactivity of Angiosperms to *Phytophthora infestans*. *Nature* **166**: 392–394.
- Müller, K. O. 1953. The nature of resistance of the potato plant to blight—*Phytophthora infestans*. *J. Natl. Inst. Agr. Botan.* **6**: 346–360.
- Müller, K. O. 1956. Einige einfache Versuche zum Nachweis von Phytoalexinen. *Phytopathol. Z.* **27**: 237–254.
- Müller, K. O. 1958a. Studies in Phytoalexins. I. The formation and the immunological significance of Phytoalexin produced by *Phaseolus vulgaris* in response to infections with *Sclerotinia fructicola* and *Phytophthora infestans*. *Australian J. Biol. Sci.* **11**: 275–300.

- Müller, K. O. (1958b). Relationship between phytoalexin output and the number of infections involved. *Nature* **182**: 167–168.
- Müller, K. O., and L. Behr. 1949. Mechanism of *Phytophthora* resistance of potatoes. *Nature* **163**: 469–471.
- Müller, K. O., and H. Börger. 1940. Experimentelle Untersuchungen über die *Phytophthora*–Resistenz des Kartoffel; zugleich ein Beitrag zum Problem der “erworbenen Resistenz” in Pflanzenreich. *Arb. biol. Reichsanstalt. Land- u. Fortwirtsch. Berlin-Dahlem* **23**: 189–231.
- Müller, K. O., J. C. Cullen, and M. Kostrowicka. 1955. Testing “true resistance” of the potato to blight, *Phytophthora infestans*. *J. Natl. Inst. Agr. Botan.* **7**: 341–354.
- Müller, K. O., and R. Griesinger. 1942. Der Einfluss der Temperatur auf die Reaktion von anfälligen und resistenten Kartoffelsorten gegenüber *Phytophthora infestans*. *Angew. Botan.* **24**: 130–149.
- Müller, K. O., J. H. E. Mackay, and J. N. Friend. 1954. Effect of streptomycin on the host-pathogen relationship of a fungal pathogen. *Nature* **174**: 878–879.
- Naef-Roth, St. 1948. Untersuchungen über den Erreger der Schrotschusskrankheit des Steinobstes, *Clasterosporium carpophilum* (Lév.) Aderh., und über den Schrotschusseffekt. *Phytopathol. Z.* **15**: 1–38.
- Neger, F. W. 1923. Beiträge zur Biologie der Erysipheen. III. Der Parasitismus der Mehltaupilze—eine Art von geduldeter Symbiose. *Flora (Jena)* **116**: 323–335.
- Newton, M., and T. Johnson. 1943. Adult plant resistance in wheat to physiologic races of *Puccinia triticina* Erikss. *Can. J. Research* **C21**: 10–17.
- Newton, R., and J. A. Anderson. 1929. Studies on the nature of rust resistance of wheat. IV. Phenolic compounds of the wheat plant. *Can. J. Research* **1**: 86–99.
- Newton, R., J. V. Lehmann, and A. E. Clarke. 1929. Studies on the nature of rust resistance in wheat. *Can. J. Research* **1**: 5–35.
- Nobécourt, P. 1927. “Contribution à l'étude de l'immunité chez les végététaux.” Lyon.
- Noll, A. 1949. Studien über die Resistenz des Weizenblattes gegen *Penicillium glaucum*. *Phytopathol. Z.* **15**: 447–481.
- Noll, A. 1950. Über anormale Kieselsäureablagerungen bei Gelbrostinfektion (*Puccinia glumarum*) bei Weizen. *Phytopathol. Z.* **16**: 483–491.
- Noll, A. 1951. Über mikroskopische Anfangssymptome der Resistenz und Anfälligkeit von Weizensorten gegen *Puccinia glumarum*. *Phytopathol. Z.* **17**: 400–405.
- Norell, I. 1954. The effect of ultraviolet light on the resistance of potato tubers to *Fusarium* species. *Physiol. Plantarum* **7**: 797–809; *Biol. Abstr.* **29**: 22522 (1955).
- Nussbaum, C. J., and C. W. Keitt. 1938. A cytological study of host-pathogen relations of *Venturia inaequalis* on apple trees. *J. Agr. Research* **56**: 595–618.
- Oort, A. J. P. 1947. Stuiifbrand specialisatie, een problem voor den Kweker. Onderzoekingen over stuiifbrand. III. *Tijdschr. Plantenziekten* **53**: 25–43.
- Paine, L. A. 1950. The susceptibility of pear trees to penetration and toxic damage by mistletoe. *Phytopathol. Z.* **17**: 305–327.
- Painter, R. H. 1951. “Insect Resistance in Crop Plants.” Macmillan, New York.
- Pantaneli, E. 1921. Sui rapporti fra nutrizione et recettività per la ruggine. *Riv. patol. vegetale* **11**: 36–64.
- Pierson, C. F., and J. C. Walker. 1954. Relation of *Cladosporium cucumerinum* to susceptible and resistant cucumber tissue. *Phytopathology* **44**: 460–465.



- Pilgrim, A. J., and M. C. Futrell. 1957. The ascorbic acid content at different stages of growth of stem rust susceptible and resistant wheats grown under different conditions. *Phytopathology* **47**: 193-195.
- Priston, R., and M. E. Gallegly, Jr. 1954. Leaf penetration by *Phytophthora infestans*. *Phytopathology* **44**: 81-86.
- Rappaport, I., and S. G. Wildman. 1957. A kinetic study of local lesion growth on *Nicotiana glutinosa* resulting from tobacco mosaic virus infection. *Virology* **4**: 265-274.
- Ray, J. 1901. Cultures et formes atténuées des maladies cryptogamiques des végétaux. *Compt. rend.* **133**: 308-311.
- Rubin, B. A., and V. A. Aksenova. 1957. The participation of the polyphenolase system in the defense reactions of the potato against *Phytophthora infestans*. *Biokhimiya* **22**: 202-208; *Chem. Abstr.* **51**: 11484 (1957).
- Rubin, B. A., and E. W. Arzichowskaja. 1948. Biochemische Charakteristik der Widerstandsfähigkeit der Pflanzen gegenüber Mikroorganismen (published by Academy of Sciences, U.S.S.R., transl. into German and publ. by Akademie-Verl., Berlin).
- Salmon, E. S. 1905. On the stages of development reached by certain biologic forms of *Erysiphe* in cases of non-infection. *New Phytologist* **4**: 217-222.
- Samborski, D. J., and M. Shaw. 1956. The effect of *Puccinia graminis tritici* Erikss. and Henn. on the respiration of the first leaf of resistant and susceptible species of wheat. *Can. J. Botany* **34**: 601-619.
- Samuel, G. 1931. Some experiments on inoculating methods with plant viruses, and on local lesions. *Ann. appl. Biol.* **18**: 494-507.
- Schilder, F. A. 1947. Zur Biologie der Reblausrassen. *Züchter* **17/18**: 413-415.
- Schmelzer, K. 1957. Untersuchungen über den Wirtspflanzenkreis des Tabakmauchevirus. *Phytopathol. Z.* **30**: 281-314.
- Schwinghamer, E. A. 1954. Physiologic specialization and the nature of parasitism in *Colletotrichum linicolum* Pethyb. Laff. L. Doctoral Thesis, Univ. Minnesota. From Eide, 1955.
- Schwinghamer, E. A. 1957. Effect of ionizing radiation on rust reaction. *Science* **125**: 23-24.
- Scott, K., A. Millerd, and N. H. White. 1957. Mechanism of resistance in barley varieties to powdery mildew disease. *Australian J. Sci.* **19**: 207-208.
- Sempio, C. 1939. Influenza delle luce e dell'oscurità sui principale del parassitamento. *Riv. patol. vegetale* **29**: 1-69.
- Sempio, C. 1942. Contributo alla conoscenza del meccanismo della resistenza in dotta dal Cadmium nei tessuti del frumento. *Ann. fac. agrar. regia univ. Perugia* **1**: 3-6.
- Sempio, C. 1950a. Metabolic resistance to plant diseases. *Phytopathology* **40**: 799-819.
- Sempio, C. 1950b. Difesa, predisposizione et malattia intese come squilibri funzionale (con particolare riguardo a studi compiuti sul frumento). *Phytopathol. Z.* **17**: 287-292.
- Shaw, M., and A. R. Hawkins. 1958. A preliminary examination of the level of free endogenous indoleacetic acid in rusted and mildewed cereal leaves, and their ability to decarboxylate exogenously supplied radioactive indoleacetic acid. *Can. J. Botany* **36**: 1-16.
- Shaw, M., and D. J. Samborski. 1956. The accumulation of radioactive substances at infections of facultative and obligate parasites including tobacco mosaic virus. *Can. J. Botany* **34**: 389-405.

- Shaw, M., and D. J. Samborski. 1957. The pattern of respiration in rusted and mildewed cereal leaves. *Can. J. Botany* **35**: 389-407.
- Shaw, M., A. Stewart, and D. R. Jones. 1954. Uptake of radioactive carbon and phosphorus by parasitized leaves. *Nature* **173**: 768-769.
- Shay, J. R., and E. B. Williams. 1956. Identification of three physiologic races of *Venturia inaequalis*. *Phytopathology* **46**: 190-193.
- Siebs, E. 1955. Untersuchungen über die Schorfresistenz von Birnen. III. Stofflicher Hinweis auf die Grundlagen der Blattschorfresistenz. *Phytopathol. Z.* **23**: 37-48.
- Silberschmidt, K. 1932. Studien zum Nachweis von Antikörpern in Pflanzen. *Beitr. Biol. Pflanz.* **20**: 105-178.
- Simons, M. D. 1954. The relationship of temperature and stage of growth to the crown rust reaction of certain varieties of oats. *Phytopathology* **44**: 221-223.
- Skoropad, W. R., and D. C. Arny. 1956. Histological expression of susceptibility and resistance in barley to strains of *Helminthosporium gramineum*. *Phytopathology* **46**: 289-292.
- Spencer, E. L., and G. L. McNew. 1938. The influence of mineral nutrition on the reaction of sweet-corn seedlings to *Phytomonas Stewarti*. *Phytopathology* **28**: 212-223.
- Sproston, T. 1957. Studies in the disease resistance of *Impatiens balsamina*. *Phytopathology* **47**: 534-535.
- Stakman, E. C. 1914. A study in cereal rusts, physiological races. *Univ. Minn. Agr. Expt. Sta. Bull.* **138**.
- Stakman, E. C. 1915. Relation between *Puccinia graminis* and plants highly resistant to its attack. *J. Agr. Research* **4**: 193-200.
- Stakman, E. C., and M. N. Levine. 1922. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. *Minn. Agr. Expt. Sta. Tech. Bull.* **8**.
- Stakman, E. C., and F. J. Piemeisel. 1917. Biologic forms of *Puccinia graminis* on cereals and grasses. *J. Agr. Research* **10**: 427-496.
- Straib, W. 1938. Über den Einfluss der Steinbrandinfektion auf das Gelbrostverhalten des Weizens. *Phytopathol. Z.* **11**: 571-587.
- Straib, W. 1940. Der Einfluss des Entwicklungsstadiums und der Temperatur auf das Gelbrostverhalten des Weizens. *Phytopathol. Z.* **12**: 113-168.
- Straib, W., and A. Noll. 1944. Untersuchungen über den Einfluss der Hitze auf den Rostparasitismus. *Zentr. Bakteriell. Parasitenk. Abt. II*, **106**: 257-277.
- Suchorukow, K. T. 1952. "Beiträge zur Physiologie der pflanzlichen Resistenz" (publ. by Academy of Sciences U.S.S.R., transl. into German and publ. by Akademie-Verl., Berlin).
- Takakuwa, M., and K. Tomiyama. 1957. The time required for the browning of midrib cells induced by the infection with two different pathogenic strains of *Phytophthora infestans* in potatoes. *Hokkaido Natl. Agr. Expt. Sta. Research Bull.* **73**: 94-99.
- Thatcher, F. S. 1942. Further studies of osmotic and permeability relations in parasitism. *Can. J. Research* **C20**: 283-311.
- Thiers, H. D., and M. B. Lester. 1949. Histological studies of bacterial blight infection of the cotton plant. *Phytopathology* **39**: 499-500.
- Tomiyama, K. 1955. Cytological studies on resistance of potato plants to *Phytophthora infestans*. II. The death of the intracellular hyphae in the hypersensitive cell. *Ann. Phytopathol. Soc. Japan.* **19**: 149-154.
- Tomiyama, K. 1956a. Cytological studies on resistance of potato plants to *Phytophthora infestans*. III. The time required for the browning of midrib of potato plants infected by *Ph. infestans*. *Ann. Phytopathol. Soc. Japan.* **20**: 165-169.

- Tomiyama, K. 1956b. Cell physiological studies on the resistance of potato plant to *Phytophthora infestans*. IV. On the movements of cytoplasm of the host cell induced by the invasion of *Ph. infestans*. *Ann. Phytopathol. Soc. Japan*. **21**: 54-62.
- Tomiyama, K., N. Takase, R. Sakai, and M. Takakuwa. 1955. Changes in the physiology of potato tuber induced by the infection of the different strains of *Phytophthora infestans*. *Ann. Phytopathol. Soc. Japan*. **20**: 59-64.
- Tomiyama, K., M. Takakuwa, N. Takase, and R. Sakai. 1956a. The influence of pre-infectional ethanol narcosis upon the physiological reaction of potato tuber to the infection of *Phytophthora infestans*. (Part 1). *Ann. Phytopathol. Soc. Japan*. **21**: 17-22.
- Tomiyama, K., N. Takase, R. Sakai, and M. Takakuwa. 1956b. Physiological studies on the defence reaction of potato plant to the infection of *Phytophthora infestans*. *Hokkaido Natl. Agr. Expt. Sta. (Japan). Research Bull.* **71**: 32-50.
- Tomiyama, K., R. Sakai, N. Takase, and M. Takakuwa. 1957. The influence of pre-infectional ethanol narcosis upon the physiological reaction of potato tuber to the infection by *Phytophthora infestans* (Part 2). *Ann. Phytopathol. Soc. Japan*. **21**: 153-158.
- Valle, E. 1957. On anti-fungal factors in potato leaves. *Acta Chem. Scand.* **11**: 395-397.
- Vavilow, N. I. 1935. The origin, variation, immunity and breeding of cultivated plants (selected writings). *Chronica Botanica* 1951, **13**: 98-103.
- Virtanen, A. I., P. K. Hietala, and O. Wahlross. 1956. Additional note on the anti-fungal factor in maize and wheat plants. *Suomen Kemistilehti* **29**: 171.
- Volk, A. 1931. Beiträge zur Kenntnis der Wechselbeziehungen zwischen Kulturpflanzen, ihren Parasiten und der Umwelt. *Phytopathol. Z.* **3**: 1-88.
- Vörös, J., Z. Király, and G. L. Farkas. 1957. Role of polyphenolase in streptomycin-induced resistance to *Phytophthora* in potato. *Science* **126**: 1178.
- Ward, H. M. 1902a. On the relations between host and parasite in the Bromes and their brown rust, *Puccinia dispersa* (Erikss.) *Ann. Botany (London)* **16**: 233-315.
- Ward, H. M. 1902b. Experiments on the effect of mineral starvation on the parasitism of Uredine fungus, *Puccinia dispersa*, on species of Bromus. *Proc. Roy. Soc.* **71**: 138-151.
- Weber, P. V. V. 1951. Inheritance of a local-lesion reaction to a mild strain of tobacco-mosaic virus. *Phytopathology* **41**: 593-609.
- Wellensiek, S. J. 1927. The nature of resistance in *Zea mays* L. to *Puccinia sorghi* Schw. *Phytopathology* **17**: 815-825.
- Wei, C. T. 1937. Rust resistance in the garden bean. *Phytopathology* **37**: 1090-1105.
- Western, J. H. 1936. The biology of oat smuts. IV. The invasion of some susceptible and resistant oat varieties, including Markson, by selected biological species of smut. *Ann. Appl. Biol.* **23**: 245-263.
- White, N. H. 1954. Infectivity of tobacco mosaic virus after inoculation. *Australian J. Sci.* **17**: 50-53.
- White, N. H., and E. P. Baker. 1954. Host pathogen relations in powdery mildew of barley. I. Histology of tissue reactions. *Phytopathology* **44**: 657-662.
- Yarwood, C. E. 1954. Mechanism of acquired immunity to a plant rust. *Proc. Natl. Acad. Sci. U. S.* **40**: 374-377.
- Yarwood, C. E. 1956. Cross protection with two rust fungi. *Phytopathology* **46**: 540-544.

- Yarwood, C. E. 1958. Heat activation of virus infections. *Phytopathology* **48**: 39-46.
- Yarwood, C. E., and L. Jacobsen. 1955. Accumulation of chemicals in diseased areas of leaves. *Phytopathology* **45**: 43-48.
- Zech, H. 1952. Untersuchungen über den Infektionsvorgang und die Wanderung des Tabakmosaik-Virus im Pflanzenkörper. *Planta* **40**: 461-514.
- Zimmerman, A. 1925. Sammelreferate über die Beziehungen zwischen Parasit und Wirtspflanze. II. Die Uredineen. *Zentr. Bakteriol. Parasitenk. Abt. II.* **65**: 311-418.
- Zoja, A. 1925. L'immunità nelle piante. *Atti. Ist. botan. Univ. Pavia* [3] **2**: 15-47.





## CHAPTER 14

# Predisposition

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## I. THE CONCEPT

The age of plants and the conditions under which they grow affect their susceptibility to disease. This environmentally conditioned susceptibility is called predisposition. More fully, predisposition is the tendency of nongenetic conditions, acting before infection, to affect the susceptibility of plants to disease. Comparable terms are "disease proneness" of Gäumann (1950), "disease potential" of Grainger (1956), "acquired susceptibility" of Raines (1922), "physiologic susceptibility," "precondition-

ing," "induced susceptibility," and "acquired disposition." Predisposed, as used here, is an internal degree of susceptibility resulting from external causes.

Other definitions or usages of "predisposition" are given by Chester (1947); Hartig (1894); Sorauer (1880); Walker (1950); and Ward (1890, 1901, 1902) in the field of plant pathology, and by Adami (1910); Arey *et al.* (1957), and Hoerr and Osol (1956) in the field of medicine. The definition used here is in fair agreement with most of these, but there are areas of difference. The principal difference is that here predisposition includes induced changes in disease proneness toward greater or lesser susceptibility, whereas predisposition as commonly defined includes only changes toward greater susceptibility. This change in definition seems a practical one. While some previous authors appear to exclude changes toward resistance in their definitions, they commonly cite changes toward resistance among their examples, or in their experimental results. Resistance and susceptibility, or increasing and decreasing resistance, or increasing and decreasing susceptibility, are different ends of the same scale, and if increase of a factor causes increased susceptibility, decrease of the same factor will likely cause increased resistance. For example, increasing levels of sugar may cause increased susceptibility of leaves to rust infections (Yarwood, 1934), and this is just another way of saying that decreasing levels of sugar caused increased resistance to rusts. Similarly, low levels of gibberellic acid decreased *Fusarium* wilt of tomatoes, while high levels decreased disease (Dimond and Corden, 1957).

Another difference between predisposition as used here and as used by some others is that commonly, especially in medicine, genetic susceptibility is included in predisposition, but here it is excluded. In human medicine, in contrast to plant pathology, genetic susceptibility is a minor factor in disease, in that different individuals, families, nationalities, and races of men are usually all susceptible to the same parasitic diseases, whereas with crop plants, each species is attacked by only a few of the many pathogens attacking crop plants, and within each species we commonly have disease resistant varieties. Therefore, genetic resistance to disease has become a major aspect of plant pathology but a minor aspect of human medicine. While genetic susceptibility could, by arbitrary definition, be appropriately included in predisposition in plant pathology, it has become of such major importance as to justify separate treatment.

Not all changes in infection which have been brought about by the host are due to predisposition. The favoring of infection by the deposition of dew on leaves in an unsaturated atmosphere due to the cooling

of leaves by radiation is presumed to act through favoring the germination of the pathogen, not through increasing the susceptibility of the host. Similarly the increased infection which is believed to result from the exudation of specific chemicals by susceptible but not by resistant hosts (Buxton, 1957) is due to a genetic character of the host, and therefore not to predisposition.

Few extensive treatments of predisposition as a biological phenomenon have been found. Sorauer (1880) discusses in detail the meaning of the term and the importance of the phenomenon. Hartig (1894) gives many examples which are valid as predisposition is here defined. However, under predisposition he also includes many other epidemiological factors, such as proximity to inoculum, whereas this topic is commonly treated under escape, production, and dispersal of inoculum. In Ward's extensive treatments of predisposition, the term seems to be used as synonymous with susceptibility and in contrast to immunity. Heald (1933) gives presence of inoculum and favorable environmental conditions for infection as examples among predisposing factors. Gäumann (1950) gives many examples of predisposition in his discussion of "changes in disease proneness due to environment." He includes treatments which act principally before, during, and after inoculation. He further divides the subject into resistance to penetration, resistance to attack, and resistance to spread.

The term predisposition is not commonly used in current plant pathology, although many examples of the phenomenon are given. Glossaries of phytopathological terms by Whetzel *et al.* (1925), the American Phytopathological Society (1940), Ainsworth and Bisby (1943), and the British Mycological Society (1950) do not include the term, but whether this is because of rarity of usage or difficulty of definition is not clear.

Environment affects disease through its direct effect on the pathogen, through its effect on the susceptibility of the host, and through its effect on the interaction of host and pathogen. The first category, while likely most important in determining the natural incidence of disease, is excluded from predisposition by definition. The second category is the subject matter of predisposition, and the third is sometimes difficult to separate from the second.

At one time in history (Whetzel, 1918), environment was considered the sole or prime cause of all animal and plant diseases. With the ascendance of the microorganism theory of disease, which is still dominant, environmental factors were relegated by most investigators to a minor or at least to a less important role. There have been many, however, who believed that microorganisms which cause or incite disease can only attack if the host has been injured or modified by the environ-



ment so as to be more susceptible to invasion by organisms which might not otherwise attack it. These people have been called the predispositionists by Whetzel in contrast to the pathogenetists, who emphasized the role of microorganisms. Greatest among the predispositionists of history have been Sorauer<sup>\*</sup> (1880) and Marshall Ward<sup>†</sup> (1890, 1901, 1902, 1905).

Whether we define susceptibility as capable of being readily attacked by a pathogen, or lacking inherent ability to resist disease, there still are several kinds of susceptibility. We could have susceptibility to penetration, susceptibility to spread of pathogen within the host (invasiveness), susceptibility to production of large numbers of reproductive units of the pathogen, susceptibility to injury, susceptibility to symptom production, susceptibility to necrosis, etc. Predisposition could be discussed in terms of the different manifestations of susceptibility (Gäumann, 1950) but here the classification of predisposition will be on the basis of cause.

Experimentally, predisposition can be clearly demonstrated only by exposing otherwise similar healthy plants to contrasting conditions, then placing them all in the same environment, and inoculating them or subjecting them to a disease-inducing environment. If the experimental pre-inoculation treatments cause differences in disease, predisposition may be said to have occurred. While predisposition has usually been demonstrated by this type of experiment, it can also be logically inferred, although not so safely, from less exacting evidence.

Much of the information on the relation of fertilizer to plant disease is derived from field trials where differences in disease were correlated with fertilizer treatments. In many of these trials the plants were exposed to fertilizer long before infection occurred, and resulting disease differences could be due to predisposition. The differences could also be due to the effect of the chemicals acting directly or through the plant on the development of the pathogen after infection, in which case no predisposition would be involved, or at least such an interpretation is questionable. In such cases it would not be practical to place all the plants in the same nutrient environment just before inoculation, and usually the experimenter was not interested in predisposition anyway. If similar fertilizers, first added just before inoculation, produced no such differences in disease, the case for predisposition would be strengthened but not established. When test nutrients are used in water cultures, it is a simple matter to grow plants in contrasting nutrient environments before inoculation and to transfer them all to the same environment at

<sup>\*</sup> Born 1839, died 1916.

<sup>†</sup> Born 1854, died 1906.

inoculation, as was done by Kendrick and Walker (1948). If differences in disease appear, predisposition may be safely inferred. Some of the evidence for predisposition cited here is of the unsatisfactory sort mentioned above and must be used with caution. Greater emphasis should be placed on properly controlled experiments.

The same cultural operation may function as a predisposing treatment in one situation and as a direct effect on the pathogen in another. In most cases, disease control with zineb is believed to be due to the toxicity of the zineb to the causal fungus (Horsfall, 1956). With reduction of smog injury by zineb (Kendrick *et al.*, 1954), the effect of zineb is presumed to be due to the effect of zineb on the host. Zineb can have profound effects on plants in the apparent absence of disease. In one field trial by the writer, 0.2% zineb spray caused the red leaves of 2 varieties of garden beet to be much greener than normal.

In another example, low soil moisture is considered a predisposing treatment in the case of *Erysiphe* on bean (Yarwood, 1949), since any effect of soil moisture on the aerial pathogen must act through the host. With *Actinomyces* on potato (Dippenaar, 1933), on the other hand, the effect of low soil moisture in increasing disease is believed to be through the effect of soil moisture on the pathogen, and therefore is not predisposition.

All basic changes in plants, from whatever cause, probably affect the susceptibility of these plants to disease. The effects are often so slight as to be unobserved. Many changes in susceptibility are missed because treatments, pathogens, dosages, and other experimental manipulations were inappropriately chosen. The most obvious effects have been discovered first, and these, within the scope of our definition of predisposition, are the subject of this chapter. In several cases incorrect observations or interpretations have been made, and some of these are difficult to avoid.

Chemical immunization (Horsfall, 1945) is a borderline subject. When a chemical increases resistance or susceptibility by changing the host, predisposition is involved. If the chemical acts to reduce or increase disease by its direct effect or that of its breakdown products on the pathogen, predisposition is not involved. The mode of action of most such chemicals is not known. Hacker and Vaughn (1957) give the control of wheat rust with cycloheximide analogues as a type preinfection resistance, yet it is known that cycloheximide is one of the most toxic of all chemicals to fungi. In the absence of further evidence, it seems more likely that the toxicity of cycloheximide to the rust fungus was responsible rather than an effect on the susceptibility of the host.

## II. THE EVIDENCE

### A. Ontogenetic Predisposition

Plants, like animals, vary in susceptibility to disease with age. Man is most susceptible to diarrhea as an infant and to gout in old age. Plants are commonly more susceptible to damping-off fungi as seedlings, to rusts at an intermediate age, and to *Rhizopus* in senescence. Four categories are commonly recognized: youth susceptibility and age resistance, youth resistance and age susceptibility, youth and age susceptibility with resistance in middle life, and youth and age resistance with susceptibility in middle life. Youth and age are, of course, only relative terms, and the absolute age is not usually known.

Examples of transition from susceptibility to resistance with age are: barberry with the basidial stage of *Puccinia* (Melander and Craigie, 1927), wheat with the uredinial stage of *Puccinia* (Chester, 1946), rye heads with *Fusarium* (Baltzer, 1930), lettuce with *Erwinia* (Erwin, 1921), peach with *Sphaerotheca* (Schnathorst and Weinhold, 1957), tobacco with *Peronospora* (Clayton, 1945), potato with *Phytophthora* (Boyd and Henderson, 1953), and peach with *Taphrina* (Fitzpatrick, 1935).

Examples of increasing susceptibility with age are: peach and apricot fruits with *Monilinia* (Roberts and Duneagan, 1932; Wade, 1956), cucumber with *Pseudoperonospora* (Doran, 1932; Iwata, 1951), lettuce with *Erysiphe* (Schnathorst and Weingold, 1957), and apple with *Botryosphaeria* (Sitterly *et al.*, 1957).

Examples of youth and age susceptibility with resistance in middle life are: certain varieties of wheat with *Puccinia* (Vohl, 1938), flax with *Sphaerella* (Loughnane *et al.*, 1946), and potato tubers with *Fusarium* (Boyd, 1952).

Examples of youth and age resistance with susceptibility in middle life are: apple with the basidial stage of *Gymnosporangium* (Giddings, 1918), potato tubers with *Erwinia* (Gregg, 1952), bean with *Uromyces* (Wei, 1937), and bean with tobacco mosaic virus (Yarwood, 1958). Other examples could be cited in each category.

There are few generalizations which can safely be made about ontogenetic predisposition except that it is common. It has been suggested (Yarwood, 1934) that susceptibility to facultative saprophytes increased with age of host tissues, whereas susceptibility to obligate parasites decreased with age. In view of the apparent reversal of age effect with *Colletotrichum trifolii* on clover (Yarwood, 1934) in comparison with *C. lindemuthianum* on bean (Yarwood, 1958), as well as

other apparent discrepancies between the results of different authors, even this generalization appears unsafe. It is likely that many factors affect the apparent trend in susceptibility with changing age of tissues.

### B. Seasonal Predisposition

Plum trees are more resistant to *Stereum* during summer months than at other times of the year (Brooks and Moore, 1926). Chestnut is more susceptible to *Nectria* in autumn and winter than in spring and summer, and spruce and silver fir are more susceptible to several wood rotting fungi during February to July than during August to January (Gäumann, 1950). Gäumann interprets the seasonal susceptibility of chestnut as due to decreased water content, and the seasonal susceptibility of spruce as due to an increased content of growth substances.

### C. Diurnal Predisposition

It is likely that the diurnal periodicities of plant pathogens are attuned to the diurnal periodicities of their hosts. Higher plants show diurnal periodicities with respect to leaf movements (Maximov, 1930), stomatal closure (Maximov, 1930), nuclear division (Maximov, 1930), elongation (Miller, 1938), organic acids in leaves (Pucher *et al.*, 1947), carbohydrates in leaves (Krotkov, 1943; Mason and Maskell, 1928), protein in leaves (Chibnall, 1924), minerals in leaves (Phillis and Mason, 1942), water content of leaves (Ackley, 1954), turgidity of leaves (Weatherley, 1951), vigor of leaves (Cox, 1954), wettability of leaves with water (Fogg, 1944), occurrence of plasmodesmata in epidermal cells of leaves (Lambertz, 1954), and guttation (Grossenbacher, 1938). It is likely that each of these periodicities could affect the susceptibility of plants to disease and thereby constitute predisposition.

Plant pathogenic fungi show diurnal periodicities with respect to the formation of spores (Weston, 1924; Carpenter, 1949; Thorold, 1955; Yarwood, 1936, 1937, 1941), discharge of spores (Murray, 1880; Hirst, 1953), germination of spores (Yarwood, 1936), and formation of appressoria (Yarwood, 1936). Each of these could be a response of the fungus to environment, and as such would have no causal relation to predisposition of the host. But each could be, in part at least, an adjustment by selection to the periodicities of their hosts. Although no example of the latter seems to have been established, there are several of diurnal differences in the susceptibility of leaves to infection.

The diurnal opening of the stomata of soybeans (Allington and Feaster, 1946) and peaches (Anderson and Powell, 1950) is correlated with the greater susceptibility of these plants to bacterial infections at



the time of maximum stomatal opening. It would be a nice story if closure of stomata could function to prevent infection with stomata-invading parasites generally, but there are at least some exceptions. Caldwell and Stone (1936) found that not only did stomatal closure not prevent stomatal entrance by *Puccinia triticina*, but that the fungus actually stimulated stomatal closure as a prelude to forceful penetration.

The greater infection following artificial inoculation with several viruses in the afternoon than in the early morning (Matthews, 1953; Yarwood, 1956a) is another example of apparent diurnal predisposition, but here no causal relation with any specific diurnal periodicity of the host has been established.

#### D. Environmental Predisposition

##### 1. Temperature

The classic example of predisposition with regard to animal diseases was reported by Pasteur *et al.* (1878). They found that chickens with a body temperature of 42° C. were normally immune to anthrax. If, however, the birds were chilled so that their body temperature was reduced to 37 to 38° C., they were highly susceptible to anthrax.

When plants are exposed to high or low temperature before inoculation, their susceptibility to several pathogens may be increased or decreased. By far the most common is an increase in susceptibility. Ward (1890) noted that if a carrot or turnip was first submerged for half a minute in boiling water before inoculation, the growth of *Botrytis* in the tissues was increased. Since the boiling water may have killed the carrot, this may be a questionable case of predisposition, since what Ward observed was the common phenomenon that killed tissues are a better substrate for many facultative parasites than are living tissues. Less equivocal cases of heat-induced susceptibility have been reported with *Botrytis* on apple (Vasudeva, 1930), *Erysiphe graminis* on *Bromus* (Salmon, 1905), *Erysiphe polygoni* on bean (Yarwood, 1956b), *Erwinia* on potato (Gregg, 1952), *Fusarium caeruleum* on potato (Boyd, 1952), *Colletotrichum falcatum* on sugar cane (Edgerton *et al.*, 1942), *C. lindemuthianum* on bean (Yarwood, 1956b) (Fig. 1), *Puccinia* on wheat (Straib and Noll, 1944), *Uromyces* on bean (Yarwood, 1956b), and with many viruses (Kassanis, 1952, 1957; Yarwood, 1952b, 1956b). The best quantitative information appears to be with viruses, where a great range of temperatures, from 6 to 96 hours at 36° C. (Kassanis, 1952) to 1 second at 55° C. (Yarwood, 1956b) have been effective. With tobacco mosaic virus on bean, the preinoculation heating may be effective when applied and discontinued as much as 4 days before inoculation.

Preinoculation heating of hosts may also decrease infection. Keyworth and Dimond (1952) found that dipping tomato roots in hot water reduced subsequent infection with *Fusarium lycopersici*. Yarwood (1956b) found that heating bean leaves for 10 seconds at 55° C. before inoculation reduced their susceptibility to tobacco mosaic virus. In both of the above cases, the hosts were obviously injured by the heat treatment, but cases of reduction in susceptibility from heat treatment without obvious heat injury are to be expected.



FIG. 1. Heat-induced susceptibility of Dwarf Horticultural beans to *Colletotrichum lindemuthianum*. These are twin primary leaves of the same plant, seeded February 2 and photographed February 27. The right leaf was heated 15 seconds at 50° C. before both leaves were inoculated at 10 a.m. February 21.

Exposure of plants to low temperatures before inoculation may also affect their susceptibility to disease, but the only clear cases found by the writer are those involving frost injury. Lasser (1938) reports that vernalization of wheat and rye reduced subsequent infection with *Tilletia tritici* and *Ustilago nuda*, respectively. Frost injury is reported to increase the susceptibility of broad beans (Moore, 1944), fig (Condit and Stevens, 1919), lettuce (Kerling, 1952), and lilies (McWhorter, 1945) to *Botrytis*; of potato tubers to *Fusarium* (Weiss *et al.*, 1928), of birch and spruce to *Nectria* (Gäumann, 1950), of larch to *Dasyscypha* (Gäumann, 1950), and of citrus to *Botrytis*, *Sclerotinia*, *Alternaria*, *Phomopsis*, *Diplodia*, and *Dothiorella* (Fawcett, 1936).

## 2. Humidity

For most fungus and bacterial diseases, free moisture is essential for the pathogen during the incubation stage, but moisture as a predisposing factor seems to be less important. In some of the cases cited, the

direct effect of moisture on the pathogen and on the susceptibility of the host may be confused.

A well-established case of moisture-induced susceptibility is the increased infection which results when the intercellular spaces of tobacco leaves are flooded with water before the leaves are inoculated with *Pseudomonas angulata* or *P. tabaci* (Clayton, 1936; Johnson, 1937; Diachun *et al.*, 1944). Here the procedure of water-soaking was discontinued before inoculation, but the leaves were still water soaked at the time of inoculation, and the increased infection could be because the intercellular water favored directly bacterial growth. Whether this should be called predisposition is debatable. As judged by the sequence of water treatment and inoculation, and the location of the water, it seems to be predisposition; as judged by the mode of action of the water on the development of the pathogen, it seems to be merely supplying a favorable environment for bacterial growth. Similar water soaking is reported (Johnson, 1947) to favor infection with several fungi, including rusts and one powdery mildew. This finding is questioned here because of the known relations of powdery mildews (Yarwood, 1957) and rusts to water. With *Uromyces phaseoli* on bean, Cohen (1951) has shown that water infiltration of the leaves increases their resistance to rust. Here the fungus germinated well on the water-infiltrated leaves, formed appressoria, but did not send infection hyphae into the water-filled substomatal cavities.

Thomas and Ark (1934) found that a high water content of the nectaries favors, or, inversely, a high sugar content of the nectaries reduces infection of pears with *Erwinia amylovora*. Under natural conditions, rainy weather or high humidity favors a high water content in the nectaries and dry weather, by favoring evaporation, causes the sugar content of the nectaries to be high.

Other cases where high water content of the tissues before inoculation is believed to favor infection are with *Erwinia* on apple twigs (Shaw, 1935) and on potato tubers (Gregg, 1952), *Pseudomonas* and *Flavobacterium* on potato (Murant and Wood, 1957), *Pseudomonas* on cucumber and peas (Riker, 1929), *Botrytis* on potato tubers (Mishra, 1953), *Oospora* on potato tubers (Allen, 1957) and *Phytophthora* on potato vines (Ward, 1890). On the other hand, reduced moisture before inoculation is believed to favor infection with *Pythium* on potato tubers (Mishra, 1953), *Peronospora* on beet (Cornford, 1953), and tobacco mosaic virus and tobacco necrosis virus on bean (Yarwood, 1955).

High soil moisture favors infection with *Botrytis* on broad bean (Wilson, 1937), with tobacco mosaic virus in tobacco and in *Nicotiana glutinosa*, with bushy stunt virus in *N. glutinosa*, with potato viruses X

and Y in tobacco (Tinsley, 1953), and intermediate soil moisture favors infection with *Corynebacterium* in tomato (Kendrick and Walker, 1948). High soil moisture reduces infection with *Fusarium* on tomato (Foster and Walker, 1947), *Erysiphe* on rye (Volk, 1931), *Erysiphe* on bean (Yarwood, 1949), and *Gibberella* on corn and wheat (Dickson, 1923).

The writer agrees with Tapke (1951) that increased infection of powdery mildews due to wilting of the leaves before inoculation, as indicated by Riviera (1924), is unlikely.

### 3. Light

Reduced light intensity before inoculation increased susceptibility of lettuce (Brooks, 1908) and tomato (Bewley, 1923) to *Botrytis*, of tomato to *Fusarium* (Foster and Walker, 1947), of elms to *Ceratostomella* (Caroselli and Feldman, 1951), of tobacco to spotted wilt virus (Samuel and Bald, 1933), of *Physalis* to potato virus Y (Ross, 1953), of beans and tobacco to tobacco necrosis virus, of tobacco to aucuba mosaic virus, and of *Nicotiana glutinosa* to bushy stunt virus (Bawden and Roberts, 1948). Short day lengths favored infection of tomato with *Fusarium* (Foster and Walker, 1947). Intermediate day lengths favored infection of currant with *Cronartium*, while short or long days reduced infection (Moshkov, 1938). High light intensity increased resistance to *Botrytis* in begonia (Sironval, 1951) and in *Lepidium* (Schmitt, 1952). Ultra-violet radiation increased susceptibility of broad bean to *Botrytis*, but had no apparent effect on the susceptibility of broad bean to *Uromyces* (Buxton and Last, 1956). X-radiation increased or decreased susceptibility of tomato to *Fusarium* depending on the dosage (Waggoner and Dimond, 1956). Exposure of potato sets to sunlight for only a few hours increased subsequent seed piece decay (Edmundson, 1939), and Isleib (1957) found that gamma radiation prevented suberization and periderm formation which processes are known to protect against seed piece decay. Schwinghamer (1956) found that gamma radiation increased the susceptibility to *Puccinia* of some moderately resistant wheat and oat varieties.

### 4. Atmospheric Pressure

Only one report of the experimental modification of disease susceptibility by manipulation of barometric pressure has been found. Bortels (1947) reports that a change from high to low barometric pressure increased susceptibility of beans, tobacco, and potato to *Pseudomonas medicaginis*, *P. tabaci*, and *Erwinia carotovora*, respectively, while the change from low to high pressure increased resistance.

Mechanical pressure on bean leaves before inoculation increased



their susceptibility to tobacco mosaic virus, tobacco ring spot virus, tobacco necrosis virus, and *Colletotrichum*, but decreased their susceptibility to *Uromyces* (Yarwood, 1953). Similar mechanical pressure after infection reduced development of *Uromyces*, *Peronospora*, and *Erysiphe*, but did not reduce virus infection.

### 5. Mineral Nutrition

There has probably been more experimentation on the effect of nitrogen, phosphorus, and potassium in disease susceptibility than on any other potentially predisposing treatments. In most cases the experiments were not planned to separate predisposition from other effects of these nutrients, but predisposition is one probable explanation of the results obtained. High nitrogen has been reported to increase susceptibility of tobacco, *Nicotiana glutinosa*, and bean to tobacco mosaic virus (Spencer, 1935; Hitchborn, 1954), of tobacco to *Pseudomonas tabaci* (Boning, 1930; Volk, 1931), of wheat to *Puccinia* (Gassner and Hassebrauk, 1931; Doak, 1954), of various plants to *Verticillium* (Donandt, 1932), of potato tubers to decay (Fehmi, 1933), and of wheat to *Erysiphe* (Schaffnit, 1922; Trelease and Trelease, 1928; Lowig, 1936). High nitrogen reduces infection of tomato by *Fusarium* (Foster and Walker, 1947), of sugar beets by *Sclerotium rolfsii* (Leach and Davey, 1942), of certain wheat varieties by certain strains of *Puccinia graminis* (Hassebrauk, 1940), and of legumes by *Agrobacterium radiobacter* (Fred *et al.*, 1932). This latter infection is not considered a disease, but illustrates the principle of predisposition as well as do infections which are usually injurious.

High phosphorus is believed to increase susceptibility of tobacco to tobacco mosaic virus (Bawden and Kassanis, 1950), of *Nicotiana glutinosa* to turnip mosaic virus (Pound and Weathers, 1953), of cucumbers to cucumber mosaic virus (Cheo *et al.*, 1952), of beans to tobacco mosaic virus (Yarwood, 1952b), and of citrus to *Thielaviopsis* (Chapman and Brown, 1942); but to increase the resistance of tobacco to *Pseudomonas* (Boning, 1930), of beets to *Phoma* (Larmer, 1937), and of tomato to *Fusarium* (Foster and Walker, 1947).

High potassium is believed to reduce infection with several species of *Puccinia* on cereals (Gassner and Hassebrauk, 1931), with *Pseudomonas* on tobacco (Boning, 1930), with *Gleosporium* on coconut (Patel and Nayar, 1936), with *Erysiphe* on wheat (Trelease and Trelease, 1928), and with *Sclerotinia fruticola* on apricots (Wade, 1956), but to increase infection with *Phytophthora* on citrus (Chapman and Brown, 1942) and *Fusarium* on tomato (Foster and Walker, 1947).

With most diseases studied, excess nitrogen is believed to favor

infection, excess potash to reduce infection, and phosphorus to be variable (Gäumann, 1950).

More important than the absolute level of separate nutrients may be the ratios of different nutrients (Alten and Ortl, 1941; Roemer *et al.*, 1938; Doak, 1954) and the concentration of all nutrients in a balanced solution (Gallegly and Walker, 1949; Walker *et al.*, 1954). A striking case of increased susceptibility with increasing nutrient concentration is with *Erysiphe* on bean (Yarwood, 1949). Since *Erysiphe* is a foliage pathogen, and since the nutrients were applied to the roots, it is clear that any effect of the nutrients on disease must have been by affecting the susceptibility of the host. It is believed that this effect with *Erysiphe* on bean was an osmotic effect rather than due to specific nutrients.

Panzer (1957b) found that when beans were germinated in mineral nutrient solutions and then transferred to distilled water and inoculated with tobacco mosaic virus, no lesions developed, while plants maintained in the nutrient solution developed local lesions. This also appears to be an osmotic effect.

Castano and Kernkamp (1956) found that all deficiencies tested—calcium, iron, nitrogen, phosphorus, sulfur, magnesium, and potassium—increased susceptibility of soybeans to *Rhizoctonia solani*. This might well be an effect on vigor, to be discussed later.

Effects of micronutrients which appear to be due to predisposition are reduction of *Erysiphe* on barley and sunflower by boron (Eaton, 1930; Schuster and Stephenson, 1940; Yarwood, 1938), reduction of *Melampsora* on flax by boron (Heggeness, 1942), reduction of *Puccinia* on wheat by boron, manganese, copper, and zinc (Ismailov, 1954), reduction of *Erysiphe* on barley by silicon (Wagner, 1940), reduction of *Erysiphe* on wheat by lithium (Spinks, 1913; Kent, 1941), reduction of *Rhizoctonia* by calcium and magnesium (Kernkamp *et al.*, 1952), and increase of *Puccinia* on wheat by zinc (Forsyth, 1957a). The foregoing must represent very delicately balanced reactions, since several investigators have failed to confirm these findings. Specifically, the effect of boron in reducing *Erysiphe graminis* on barley was not confirmed by Cherewick (1944), or Yarwood (1958). The reduction of *Erysiphe* on sunflower (Yarwood, 1938) could be demonstrated in water cultures but not in soil. The reduction of *Melampsora lini* on flax by boron could not be confirmed by Colhoun (1945) and could not be repeated at will in Minnesota, but the writer has been told the effect has been clearly demonstrated in the field on one occasion since the original observation by Heggeness.

The species of host and pathogen may be important in these nutrient effects. In the writer's trials (Yarwood, 1958), additions of lithium nitrate

and chloride to cultures in greenhouse soil increased the resistance of cucumbers to *Erysiphe cichoraccarum*, but had no clear effect on *Erysiphe polygoni* on bean. The lithium-treated, mildew-inoculated cucumbers yielded significantly more than the mildew-inoculated cucumbers without lithium.

## 6. pH

High pH of the soil is reported to favor infection by *Thielaviopsis* on citrus (Chapman and Brown, 1942), *Erysiphe polygoni* on cowpea (Brown, 1930), and *Erysiphe graminis* on wheat (Schaffnit and Meyer-Hermann, 1930), but low pH is believed to favor *Fusarium* on tomato (Foster and Walker, 1947) and *Botrytis* on broad bean (Wilson, 1937). No effect of pH on infection with *E. polygoni* on clover was detected by the writer (Yarwood, 1958).

It seems clear that mineral nutrients and pH may have profound effects on disease, but that these results are difficult to repeat or interpret.

The differences in the susceptibility of wheat to *Tilletia tritici* as determined by the environment where the seed was grown (Holton and Heald, 1936) is apparently a type of environmental predisposition, which cannot now be interpreted in terms of any specific environmental factor.

## E. Wounding Predisposition

When plants are wounded before inoculation, infection with many plant pathogens is affected, usually favored, and wounding, therefore, constitutes a type of predisposition. When wounding is part of the inoculation process, as in most artificial inoculations with viruses, the term predisposition can be applied less safely. Wounding before inoculation favors infection with *Stereum* on plums (Brooks and Moore, 1926), with *Penicillium* on gladiolus (Lauritzen and Wright, 1934), *Fusarium* on potatoes (Weiss *et al.*, 1928), *Fusarium* on beans (Burke and Seliskar, 1957), *Botrytis* on grapes (Wilhelm, 1944), *Podosphaera* on apples (Berwith, 1936), *Erysiphe* on bean (Fig. 2), *Colletotrichum* on bean (Yarwood, 1958), *Gnomonia* on sycamore (Schuldt, 1955), with several fungi on harvested vegetables (Rose *et al.*, 1939), and with canker-producing pathogens on hops (Yarwood, 1951b). Occasionally, wounding may reduce susceptibility, as with *Fusarium* on tomato (Keyworth and Dimond, 1952). Wounding is believed to favor infection by making a break in a mechanical barrier which obstructs the pathogen, but in many cases the situation is more complex.

Browning (1954) found that when inoculated leaves of susceptible varieties of oats were detached after inoculation with *Puccinia*, their reaction to rust was not changed greatly. When leaves of certain varie-

ties, inoculated with rust strains to which they were normally resistant, were detached and placed on sugar solution, they became susceptible. Forward (1957) and Forsyth (1957b) found a similar phenomenon with *Puccinia* on wheat.

When leaves of *Nicotiana glutinosa* are rubbed and later inoculated with tobacco mosaic virus without further wounding, much less infection normally results than if the inoculum is applied simultaneously with the wounding process (Holmes, 1929). When this is examined more carefully, however, (Jedlinski, 1956) it is found that there is a period of about 10 minutes during which the susceptibility of the wounded leaves

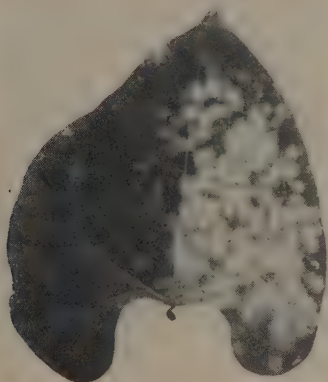


FIG. 2. Effect of carborundum treatment of the primary leaf of Black Blue Lake bean on its susceptibility to powdery mildew. Seeded July 19, photographed August 15. The right upper side of the leaf was rubbed for 10 brush strokes with dry carborundum before inoculation with conidia of *Erysiphe polygoni* at 11 a.m. August 2.

to virus actually increases. Jedlinski's results have been confirmed and extended with tobacco mosaic virus on bean (Yarwood, 1958). It is found that if inoculations are properly timed from 0 to 100 minutes after wounding, there is an initial decrease in susceptibility within 1 minute, followed by an increase to a second maximum at about 8 minutes, which is in turn followed by a decrease to zero at about 100 minutes.

In inoculations of sweet potatoes with *Rhizopus*, more infection resulted when roots were wounded by pressure, with no apparent break in the skin, than when the skin was removed with a sharp knife so as to leave no crushed tissue (Yarwood, 1958). Here it would appear that the principal effect of the wounding was to increase the susceptibility



of the tissues to the continued invasion by the fungus, rather than to expose the tissue to initial contact with the pathogen.

An unusual type of predisposition by wounding has been analyzed in detail by Braun (1952). He found that healing for 48 hours after an initial wounding favored later infection with *Agrobacterium tumefaciens* on *Kalanchoe* stems.

The removal of plant parts is a type of wounding and also a means of changing the physiology of the plant, especially the carbohydrate balance. The wounding and the change in carbohydrate balance may function independently as predisposing factors. With *Pseudomonas savastanoi* on olive (Hewitt, 1938) and *Nectria galligena* on apple (Crowdy, 1952), the function of the removal of host leaves is apparently to provide entry points for the pathogen. In the following cases the removal of plant parts is believed to affect disease by changing the carbohydrate balance of the plant. With *Macrosporium* on tomato, removal of fruit or removal of growing points and young leaves increased the resistance of the remaining parts, but general removal of foliage increased susceptibility (Rowell, 1953; Horsfall and Dimond, 1957). Similarly removal of leaves of young elm trees in June increased their susceptibility to *Ceratostomella* (Zentmyer *et al.*, 1946). With *Verticillium* wilt, removal of host leaves is reported to decrease disease in tomato (Roberts, 1944), but removal of flowers and buds is reported to decrease the disease in cotton (Suchorukov, 1957). These latter two cases are difficult to reconcile because removal of leaves in one case and flowers and buds in the other are expected to have opposite effects on the carbohydrate balance of the plants.

#### F. Chemical Predisposition

Chemicals applied to plants before inoculation with pathogens commonly reduce infection due to the toxicity of the chemicals for pathogens. This phenomenon is responsible for most of the extensive literature on fungicidal control of plant diseases and is not clearly related to predisposition. However, there are many cases where the disease reducing or increasing power of a chemical is not clearly associated with any direct toxic or stimulatory action of the chemical on the pathogen. These are included here under predisposition, because it is believed that these chemicals act through their effects on the host.

Copper increases the infection of potatoes with *Penicillium* (Dillon Weston and Taylor, 1944), of potatoes with *Fusarium* (Newton, 1952), of oranges with *Diplodia* (Hopkins and Loucks, 1946), of celery with *Anatospora* (Newhall, 1944), and of beans with tobacco mosaic virus (Yarwood, 1954b). In all these cases, copper, rather than serving as a

nutrient (see above), was applied at concentrations believed to be toxic to the pathogen and the host. The increased infection can be best explained as predisposition due to host injury, which was dominant over the effect of copper on the pathogen.

When potato tubers are cut and planted, the normal process of wound healing is usually adequate to protect the seed pieces from the saprophytic organisms in the soil. If the cut tubers were treated with solutions of salts of copper, cobalt, nickel, or iron, however, the process of wound healing was delayed, *Penicillium* developed abundantly on the cut surfaces, many such pieces decayed, and emergence of shoots from the cut tubers was reduced (Dillon Weston and Taylor, 1944; Werner, 1938; Sanford, 1951; Newton, 1952).

Other examples of increased susceptibility from the use of pesticidal chemicals are as follows: *Claviceps* on wheat from use of 2,4-D (Longchamp *et al.*, 1951); *Erysiphe* on *Bromus* from the use of ether, alcohol, or chloroform (Salmon, 1905); *Erysiphe* on barley from use of herbicidal oils (Crafts and Reiber, 1948); *Puccinia* on wheat from the use of ether or chloroform (Stakman, 1914; Gassner and Hassebrauk, 1938), 2,4-D (Lyles *et al.*, 1957), maleic hydrazide (Samborski and Shaw, 1957), zinc, manganese, and copper (Forsyth, 1957a), and DDT (Johnson, 1946); *Fusarium* on tomato from a variety of organic chemicals (Davis and Dimond, 1952, 1953); *Alternaria* on cauliflower from use of alcohol (Minkevicius, 1932); *Peronospora* on broccoli from use of emulsifiers (Natti *et al.*, 1956); *Agrobacterium* on cherry from the use of dichlone (Young and Deep, 1956); *Didymella* on tomato from the use of 2:4:6-T (Croxall *et al.*, 1957), and tobacco mosaic virus on bean from the use of zinc and silver (Yarwood, 1954b). The respiration inhibitors, 2,4-dinitrophenol, thiourea, and sodium fluoride broke down resistance to *Fusarium* in tomato (Gothoskar *et al.*, 1955). It is probable that concentration of test chemicals (Dimond and Corden, 1957) and other factors are important in explaining apparently contradictory results of different investigators, as for example the different effect of 2,4-D on wheat rust as observed by Ibrahim (1951) and Lyles *et al.* (1957).

### G. Vigor Predisposition

Vigor of the host is commonly believed to be an important factor in predisposition to disease, but much of the information is difficult to interpret. Many of the above effects of specific treatments on disease have been and will again be attributed to the effect of these treatments on vigor. In the present section, aspects of host vigor not readily classified elsewhere will be treated. Vigor in plants is a useful term but one hard to define. We might say that a vigorous plant is one in which the

dry weight is increasing rapidly. In very few cases have the effects of treatments which predispose plants to infection been correlated with the effects of these treatments on the production of dry weight. Nevertheless, much useful information has accumulated.

Following are some examples which bear on the question of vigor and disease: rapidly growing tobacco plants become more severely infected by *Pseudomonas angulata* (Wolf, 1957), infection with *Botrytis* is favored on the yellow leaves of lettuce (Brooks, 1908), senescent or necrotic tissue favors *Botrytis* infection on various hosts (Baker, 1946), infection with *Pseudopeziza* is favored by poor growth of currant and gooseberry (Blodgett, 1936), large potatoes and sugar beet plants are more liable to virus infection than small ones (Bawden, 1950), active carbon assimilation favors infection with obligate parasites (Butler and Jones, 1949), vigorous celery plants favor infection by *Septoria* (Thomas, 1921), slow growing bean plants are more susceptible to *Erysiphe* (Townsend, 1939), reduced turgor of poplar favors infection with *Dothichiza* (Butin, 1957), more infection with tobacco mosaic virus on rapidly growing than on hardened *Nicotiana glutinosa* plants (Samuel and Bald, 1933), and no relation of host vigor to *Fusarium* infection of tomato (Foster and Walker, 1947). The difficult problem of host vigor and disease susceptibility is discussed by Raines (1922), Gäumann (1950), Tapke (1951), and many others.

In certain types of experiments, a positive correlation between vigor and disease is indicated, and in others the reverse or no relation is indicated. Perhaps the most striking and also the most controversial results have been secured with powdery mildews. For example, Tapke (1951) studied the effect of preinoculation environment on infection of barley by *Erysiphe graminis*. He grew plants in three contrasting environments—outdoors, in the greenhouse with light watering, and in the greenhouse with liberal watering. After several weeks he placed them all in the same environment and inoculated them. If we accept the size of his photographed plants as an index of vigor, then plant vigor increased in the order given. As mildew infection also increased in the order given, we have here an excellent positive correlation between vigor and disease, which is about what Tapke concluded.

On the other hand, Trelease and Trelease (1928) produced contrasting differences in growth of wheat plants by the mineral nutrients  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{MgSO}_4$  in water culture. They measured the dry weight of the healthy and mildewed plants and calculated mildew injury on the basis of the relative weights of healthy and mildewed plants with the same nutrients. While these experiments have minor imperfections, they certainly provide one of the most comprehensive sets

of data on the relation of vigor to disease. While the nutrient treatments caused great differences in dry weight and great differences in damage from mildew, the authors conclude that under their conditions host vigor did not appear to determine susceptibility to mildew.

In a study of the relation of soil moisture and nutrient concentration to infection with *Erysiphe polygoni*, Yarwood (1949) followed the green weight of healthy and diseased plants. At low soil moisture with the smallest green weight for the healthy plants, mildew reduced green weight by about 80%. At intermediate soil moisture, with intermediate green weight for the healthy plants, mildew reduced green weight by about 65%. At high soil moisture with heaviest healthy plants, mildew reduced green weight by about 30%. In the soil moisture series there was thus an inverse relation between vigor and mildew. With beans in nutrient solutions ranging from 0.25 to 4 times the concentration of nutrients in standard Hoagland solution, the green weight of healthy plants increased with 0.25 to 1 times Hoagland solution. Mildew severity, on the other hand, increased fairly uniformly through 0.25, 0.5, 1, 2, and 4 times Hoagland solution. Here there was a positive correlation of mildew and host vigor as the nutrient concentration increased from 0.25 to 1 times Hoagland solution, and a negative correlation as the nutrient concentration increased from 1 to 4 times Hoagland solution.

Recent champions (Howard, 1940; and Bromfield, 1948) of the idea that plants fertilized with manures and composts tend to resist disease have attracted many followers among nonprofessional gardeners, but less support from professional agriculturists. Their ideas can be classified here under host vigor, as there is no doubt that composts and manures promote vigorous plant growth. Sir Albert Howard is a true predispositionist when he writes: "Insects and fungi are not the real cause of plant diseases but only attack unsuitable varieties or crops imperfectly grown." Since he reports no inoculations with any pathogen, it is difficult to assail his evidence. Most plant pathologists apparently consider it below their dignity to answer these preposterous claims, but Wager (1945) has performed a real service in experimentally exploring the relation of compost to disease. He showed that tomatoes grown with chemical fertilizers in one case and with 20 tons per acre of compost in another were about equally infected with and damaged by *Pseudomonas solanacearum* and *Heterodera marioni*.

A clear relation of host vigor to disease has been demonstrated with detached leaves, but the relation of this to field severity of disease is not so clear. Detached leaves decrease in dry weight when floated on water in the dark, but may increase in dry weight and longevity on adequate concentrations of sucrose or other sugars (Yarwood, 1934, 1946). Sus-



ceptibility of clover leaflets to the obligate parasites *Erysiphe* and *Uromyces* was favored by high concentrations of sucrose, whereas infection with the facultative saprophytes *Colletotrichum* and *Macrosporium* was favored by water or by low concentrations of sucrose.

Sugar content of tissues may be involved in the effects of senescence, light, and growth substances on infection with *Botrytis*. *Botrytis cinerea* commonly attacks senescent tissue, where the sugar content is presumably low (Baker, 1946). *Botrytis* infection sets in earlier on cabbage leaves kept in darkness, than on cabbage leaves kept in light (Suchorukov, 1957). Direct addition of sucrose to the substrate of detached broad bean leaves reduced the size but not the number of *Botrytis* lesions (Yarwood, 1958). The sugar content of healthy tissue of diseased grapes was greater than that of healthy grapes (Nelson, 1951). Phenoxy acids reduced the size but not the number of *Botrytis* lesions on broad bean (Crowdy and Wain, 1950). In trials by Mostafa and Gayed (1956), 2,4-D reduced the sugar content of broad bean leaves and reduced the area of *Botrytis* infection per leaf, but the separate effects of 2,4-D on numbers and size of lesions is not stated. Since growth substances such as maleic hydrazide and 2,4-D have profound effects on the sugar content of tissues (Naylor, 1951; Mostafa and Gayed, 1956), the effects of these chemicals on sugar content may explain in part their effects on disease (Horsfall and Dimond, 1957), but such an interpretation seems difficult to reconcile with more direct effects of sugar on *Botrytis* infection.

With viruses as a group, the interrelation of sugar content of tissues, vigor, and susceptibility is also not clear, although very striking effects have been demonstrated. Panzer (1957a) and Yarwood (1952a) indicate a negative relation of sucrose concentration of the substrate to the formation of local lesions of tobacco mosaic on bean under certain conditions, but Leben and Fulton (1951) indicate that sucrose in the substrate favored lesion formation by tobacco ring spot virus on cowpea. Yarwood (1952a) has suggested that low carbohydrate level of the leaves might favor infection in virus-host combinations when necrosis results but that the reverse might apply to infections where no necrosis results. Here, as with *Botrytis* infections, numbers and size of lesions may be differently affected by the same treatment and this may account for the apparent disagreement between investigators. High carbohydrate level clearly decreases the size of the lesions but may simultaneously increase the number of lesions. (Yarwood, 1952a). The effect is further complicated by the reduced humidity around leaves in closed chambers when sucrose solution is used rather than water, since it has been shown (Yarwood, 1955) that reduced humidity favors virus infection. Further, there

is an apparent contradiction, as far as the carbohydrate interpretation is concerned, in the increased infection following preinoculation dark treatments resulting in low carbohydrate levels and the increased infection from afternoon inoculations when the carbohydrate level is highest (see light and diurnal effects above). The difficulties of reconciling the contrasting effects of sugar infiltration of leaves, preinoculation darkening of leaves, preinoculation heating of leaves, diurnal periodicity of susceptibility of leaves, season of growth, age of leaves, blocking of stomata, 2,4-D treatment of leaves, and water content of leaves, with the carbohydrate interpretation of the susceptibility of beans to tobacco necrosis virus are discussed by Wiltshire (1957).

Changes in turgor of cells are undoubtedly related to vigor. Reduced turgor could result from reduced water content of tissues, from high osmotic pressure of external solutions, from prior infection, and from other causes. Reduced turgor is reported to increase susceptibility of *Eucharis* to *Botrytis* (Brown and Harvey, 1927) of poplar to *Dothichiza* (Butin, 1957), of wheat to *Erysiphe* (Riviera, 1924), and to increase or decrease susceptibility of beans to tobacco mosaic virus and tobacco necrosis virus (Yarwood, 1955). In the latter example, wilting of leaves before inoculation increased infection if the water deficit was 0 to 15% of the original green weight of the leaves, but decreased infection if the water deficit were greater than this. Wilting of the leaves after inoculation, on the other hand, always increased infection. From these experiments it would seem that the major effect of wilting was to increase the internal susceptibility of the tissue. The reduced susceptibility from severe wilting before inoculation could have been because flaccid cells were less easily punctured by the inoculation procedure.

### H. Grafting Predisposition

It is generally believed that the two components of a graft retain their specific susceptibilities to plant pathogens indefinitely after union (Leach, 1929; Scheffer, 1957), although the over-all gross response of the resulting plant may be changed (Bennett and Costa, 1949). In some cases, however, the vigor and growth of the scion may be reduced as a result of grafting, and this may reduce disposition to disease. Gäumann (1950) gives as examples *Gymnosporangium* of pear on quince root and *Cladosporium* of tomato on *Datura* roots. Another special case is that of buckskin virus on sweet cherry. Here Rawlins and Parker (1934) have shown that Mahaleb rootstock in some way causes the sweet cherry to escape or resist natural infection. Even one *Prunus avium* root apparently confers susceptibility on a tree.

### I. Prior Infection

A fungus or virus may predispose tissue to itself or to other pathogens. A classic interpretation of the pathology of *Monilinia* and *Sclerotinia* is that these fungi release pectic enzymes which diffuse ahead of the fungus and predispose the tissue for further advance of the same fungus (Valleau *et al.*, 1933; Brown, 1936). *Dothichiza* on poplar may predispose tissue to itself by release of toxins (Butin, 1957).

Cases where infection with one pathogen may predispose to infection by a nonrelated pathogen are: *Peronospora* to bacteria in cabbage (Fulton and Walker, 1946); *Uncinula* to *Botrytis* on grape (Boubals *et al.*, 1955); *Taphrina* to *Monilinia* on peach (Mix, 1930); *Taphrina* to *Sphaerotheca* on peach (Yarwood, 1939); *Uromyces* to *Erysiphe* on bean (Yarwood, 1957); *Tilletia* to *Uromyces*, *Fusarium*, and *Helminthosporium* on wheat (Fischer and Holton, 1957); *Phytophthora* to *Fusarium* on potato (Gäumann, 1950); *Rotylenchus* to *Fusarium* on cotton (Neal, 1954; Martin *et al.*, 1956); *Thomasiniana* to *Leptosphaeria*, *Fusarium*, and *Didymella* on raspberries (Pitcher and Webb, 1949); and *Uromyces* to tobacco mosaic virus on bean (Yarwood, 1951a).

Prior infection may also reduce infection with a later pathogen. Examples are: *Phytophthora* following viruses X and Y in potato (Müller and Monro, 1951); cucumber mosaic virus following *Erysiphe* on cucumber (Blumer *et al.*, 1955); *Erysiphe* following *Tilletia* on wheat (Sempio, 1938); *Uromyces* following *Uromyces* on bean (Yarwood, 1954a); aucuba mosaic virus following tobacco mosaic virus on tobacco (Kunkel, 1934); and *Rhizobium* following *Rhizobium* on legumes (Fred *et al.*, 1932). The phenomenon of acquired immunity or cross protection, a special case of predisposition best documented with viruses, will be treated in more detail in another section of this volume.

### J. Predisposition and Nonparasitic Diseases

Predisposition to physiologic diseases is basically similar to predisposition to parasitic diseases. Any treatment which alters the basic physiology of the plant may influence its response to some injurious influence. Recorded examples are: growing plants in shade predisposes them to sun injury (Hartig, 1894); low potassium predisposes potato tubers to wound injury (Boyd and White, 1956); high soil moisture predisposes plants to smog damage (Koritz and Went, 1953); rust infection (Yarwood and Middleton, 1954) (Fig. 3) and zineb applications (Kendrick *et al.*, 1954) protect against smog damage; rust infection increases winter killing (Chester, 1946); and high soil moisture, growth at high temperature, rapid growth, low potassium, and narcosis predis-

pose plants to frost damage (Levitt, 1941). Kerling (1952) reports that plants grown under sterile conditions suffered little from frost or *Botrytis* alone, but a combination of the two proved lethal.

There is a diurnal periodicity in the tolerance of plants to heat (Laude, 1939; Van Arsdel *et al.*, 1956; Yarwood, 1958) and presumably also to cold, since heat and cold resistance are correlated (Coffman, 1957).



FIG. 3. Effect of rust infection on susceptibility of beans to smog injury. Plant on right was inoculated with *Uromyces phaseoli* February 27. Both were equally exposed to artificial smog for 8 hours on March 3. Photographed March 4. The rust infection protected the plant on the right from smog damage. From studies of Yarwood and Middleton (1954).

### III. IMPLICATIONS AND DISCUSSION

#### A. Interpretation of Effects

It is always desirable to refer effects to basic causes, otherwise much information consists of empirical observations and correlations, and is less well understood. Copper treatment of cut potatoes is followed by growth of *Penicillium* and other fungi on the cut potatoes, and later by seed piece decay (Dillon Weston and Taylor, 1944). Control seed pieces showed much less growth of fungi and much less seed piece decay. It is reasonably certain that the copper did not directly stimulate the growth of the fungi or directly cause seed piece decay. It is believed that the copper prevented the normal periderm formation and suberization of



the cut tubers, that in the absence of normal suberization the nutrients in the potato stimulated the growth of fungi naturally present, and that these fungi invaded the seed pieces and caused their decay. Even this apparently logical interpretation is probably inadequate. Dillon Weston and Taylor believed that the fungus growth was greater than could be explained by the phytotoxic action of the copper and the prevention of suberization. It is likely that in the future the basic cause of this copper effect will be explained still further, and perhaps we may learn that copper favors seed piece decay by suppressing certain enzymes necessary for suberization and periderm formation, or that these processes are at least partially independent.

Similarly when preinoculation darkening of plants favors virus infection (Samuel and Bald, 1933; Bawden and Roberts, 1948), it is believed that the treatment exerted its influence through some secondary effect of the light. Increased nitrogen as a result of darkening of the plants has been correlated with the increased susceptibility (Watson, 1953) but it is probable that the basic interpretation of the effect of preinoculation darkening is more complex.

The effects of defoliation, light, growth substances, boron, and sugar on fungus disease development have been nicely integrated by Horsfall and Dimond (1957). Here the key to the interpretation is the sugar content of the tissues, and defoliation, light, growth substances, and boron are interpreted in terms of their effects on the sugar content of the tissues.

The increase in susceptibility of wheat to rust which is induced independently by DDT, maleic hydrazide, zinc, manganese, and cobalt, and by detachment of leaves (Forsyth, 1957a, b), may be all due to the same basic cause, such as increased amino acids or sugars.

Slow growth (Townsend, 1939) and low soil moisture (Yarwood, 1949) are reported to predispose beans to powdery mildew. Low soil moisture is a major cause of slow growth. Certainly the effect of soil moisture on bean mildew must be an indirect one, as the mildew fungus is well removed from the soil, whereas slow growth is specifically manifest in the leaves where the pathogen develops. Likely, other causes of poor growth, such as certain types of killing of roots, are, from the point of view of the leaves, similar to low soil moisture in their effect on leaf growth and can be expected to increase susceptibility to powdery mildew. It seems plausible, therefore, that slow growth is a more direct predisposing factor for mildew susceptibility than is low soil moisture. But, if true, this has only pushed back primary causes one step further. It is obviously the condition in the leaf epidermal cells which is the direct determining factor in mildew susceptibility, and this, as a cause

of mildew susceptibility, is largely unknown, although osmotic pressure in the epidermal cells has been suggested as a primary factor.

The increased susceptibility of plants to infection as a result of frost, heat, narcotization, and mechanical injury (see above) may be basically similar in that any of a variety of injuries would have produced the same effect. The more basic cause of the increased susceptibility might be prevention of suberization, release of nutrients, or a variety of reasons. The multiplicity of empirical treatments which will produce changes in disease resistance, is likely much greater than the basic causes of the changes.

While it is desirable to refer known changes in susceptibility to basic causes, this is usually impossible now. In this discussion, most of the responses reported must be referred to the treatments with which they are associated. Much more library work of the type of Horsfall and Dimond (1957), and even more of laboratory work, will be necessary before even the present knowledge of predisposition can be properly integrated. The basic causes of predisposition are many, and in many cases are probably the same as the chemical bases of genetic susceptibility and resistance, and like them, are largely unknown.

### B. Uses in Disease Control

All knowledge of factors affecting the incidence of disease, including predisposition, has potential value in disease control. At present, however, exclusion, eradication, protection, therapy, and genetic immunization, mainly directed at the pathogen, constitute the major methods of disease control. Predisposition, directed at the host, plays little part at present, and without further basic advances in knowledge, is not likely to play an important part in the near future. Among the few cases where predisposition may be a factor in present disease control are: control of *Rhizopus* rot of sweet potatoes by avoidance of bruises on the harvested crop (Walker, 1950); control of *Fusarium* wilt in wilt resistant varieties of cotton by control of the nematodes (Martin *et al.*, 1956); control of fire blight of pears by reduced nitrogen fertilization (Anderson, 1956); control of southern *Sclerotium* rot of sugar beet by increased nitrogen fertilization (Leach and Davey, 1942); control of cherry buckskin virus by grafting sweet cherries on Mahaleb rootstocks (Rawlins and Parker, 1934); and control of smog damage by zineb sprays (Kendrick *et al.*, 1954).

Most crops, during the course of their life cycle, are in potential danger of being destroyed by any one of several diseases. Yet disastrous epidemics, even in the absence of disease control, are relatively rare. For most successful crops the relatively low incidence of specific diseases is

usually because of the scarcity of the pathogen. If the pathogen is abundant, scarcity of disease is usually because the environment has been unfavorable for infection. Rarely is low incidence of disease caused by physiologic resistance of the host.

It is a disturbing commentary on our knowledge of plant pathology that most known cases of predisposition, especially those involving treatments which offer promise of increased crop productivity, usually result in increases in susceptibility. The many hopes in the past of growing plants in such a way that they will resist disease, have mostly proved illusory.

Most recorded predisposing treatments have increased the susceptibility of plants which were genetically somewhat resistant. There are fewer cases where predisposing treatments have greatly increased resistance of plants which were genetically rather susceptible, yet these are what is needed in order to apply predisposition to control.

### C. Predisposition and Disease Type

The known degree of predisposition varies with different types of pathogens. With wound pathogens and with nonobligate parasites, the potential predisposition is believed greater than with pathogens which enter without wounds and with obligate parasites, but the evidence is not clear. Hiley (1919) and Hubert (1931) believe that whether one is a pathogenetist or a predispositionist depends on the type of parasite one is working with. Students of the pathology of *Dasyscypha willkommii*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, or *Colletotrichum lindemuthianum* would presumably be predispositionists. With *Dasyscypha* on larch (Hiley, 1919), the facultatively parasitic fungus is normally present; whether it becomes pathogenic or not depends on wounds and on the vigor of the tree. With *Sclerotinia* and *Botrytis* on apricot (Smith, 1931; Yarwood, 1948), one or the other of these fungi are normally present on the blossoms, and whether or not jacket rot results depends on weather conditions, which in turn are believed to act both on the susceptibility of the host and directly on the growth of the pathogen. The presence of a senescent receptacle is believed essential for fruit infection. The greatest quantitative increase in infection due to heat predisposition as shown in studies made by the writer is with the facultative saprophyte *Colletotrichum* on bean (Yarwood, 1956b). Treating leaves for 10 seconds at 50° C. before inoculation increased the number of lesions elevenfold in one test, which increase was greater than with any other of 2 rust fungi, 1 powdery mildew fungus, and 4 viruses tested.

A student of the rusts or downy mildews would likely be a pathogenetist, since the presence of these fungi usually denotes disease, which disease is less likely to be affected by the predisposition of the host than in the previous examples.

The most striking cases of predisposition are with viruses. Viruses are regarded as obligate parasites, and unique among the obligate parasites of higher plants, viruses are also wound parasites. The great response of viruses to predisposing treatments applied to the host is more likely because of their characteristic of wound entry than because they are obligate parasites, since the obligately parasitic downy mildews, powdery mildews, and rusts show no greater response to predisposing treatments than do the nonobligate parasitic fungi and bacteria. The wounding required for entry of viruses may itself function as a predisposing treatment but it is difficult to separate the entry function of the wound from the changed susceptibility as a result of wounding. It is clear, however, that the entry of virus into the lumen of cells, where it is known to later reach a high concentration, is not enough to bring about virus multiplication or disease development. The many attempts to inoculate with plant viruses by injecting the virus into individual cells, when it is known that many thousands or even millions of virus particles are introduced, have been mostly unsuccessful, although it is reliably believed that plant virus lesions normally originate from one particle. It therefore seems likely that the success in bringing about infection by rubbing leaves with virus plus abrasive is due to accidentally placing the virus at a specifically favorable site for multiplication in the cells, or to an effect of the rubbing in predisposing the cells to susceptibility, or both. While there is no conclusive evidence on these points, I favor the view that rubbing predisposes the cells to infection. Two items which may support this are the pressure effect and the extra-rubbing effect. When bean leaves are pressed dry before inoculation (Yarwood, 1953), it seems likely that no, or few, wounds which would favor entry of virus are made in the outer cell wall, yet such pressed leaves yield more lesions when later inoculated by rubbing than do unpressed leaves. This pressure is regarded as a predisposing treatment, and since ordinary inoculation by rubbing is necessarily done with slight manual pressure, it seems that pressure must function as a predisposing treatment in ordinary inoculations. Could it be that rubbing with abrasive removes the cuticle in places, that where the cuticle is removed the plasmodesmata are exposed, that the pressure of rubbing forces cell sap to the surface through the plasmodesmata, that the cell sap mixes with the virus inoculum, that when the pressure is released the virus is drawn



into the cells through the plasmodesmata, and the virus on the surface of the plasma membrane of the relatively uninjured cell is in a position to increase and bring about infection?

Rubbing bean leaves with a wet brush without virus for one or more strokes before or after rubbing inoculation decreases infection (Yarwood, 1955). It is hard to imagine how this rubbing could have greatly reduced the virus in the cells; therefore, it appears that the extra-rubbing reduced the susceptibility of the cells. In one situation I am suggesting that rubbing increased susceptibility, and in another that rubbing decreased susceptibility. This apparent inconsistency might be reconciled by the observed rapid change in the susceptibility of cells after wounding (see treatment of wounding above) first toward greater resistance, then toward greater susceptibility, and then toward greater resistance again.

Other causes of predisposition for virus infections which are not known to apply to fungus infections are the increased susceptibility due to phosphate and sulfite in the inoculum, the increased susceptibility due to diurnal changes in the susceptibility of the host, and the increased or decreased susceptibility due to wilting. These and other predisposing treatments mentioned above, may be related. It is interesting that wounding, keeping plants in darkness, and high temperature favor protein hydrolysis and favor susceptibility to viruses, but there is no good reason to believe there is a causal relation in this correlation.

#### D. Genetic Susceptibility and Predisposition

It is desirable to consider that genetic susceptibility and resistance have their counterparts in physiologic susceptibility and resistance, here called predisposition. In other words, genetic susceptibility and susceptibility due to predisposition may be due to the same chemical or physiological cause. Of course, there may be several biochemical bases for predisposition in the same species or variety, and the likelihood of the case of genetic and the case of physiologic susceptibility chosen being due to the same cause in a given study is rather remote. There are not enough cases where the chemical nature of genetic resistance and the chemical nature of physiologic resistance are known, to explore the above hypothesis. A semi-hypothetical case will be used. The presence of protocathechuic acid and catechol in the dead outer scales of colored and smudge-resistant onions (Walker, 1950) is one of the few cases where genetic resistance is known to be due to a specific chemical. The degree of resistance of these varieties, however, varies greatly in different years (Hatfield *et al.*, 1948) and it appears highly suggestive that the

year-to-year variation in resistance of the colored varieties is greater than of the white varieties. It seems safe to predict that the amount of protocatechuic acid in onions may be greatly influenced by treatments applied to the growing crop. If so, we would have here a case where genetic and physiologic resistance and susceptibility could have the same biochemical basis.

Similarly, high or low acidity of host cells, thickness and toughness of cuticle, reaction of cells by sudden necrosis which thereby restricts the pathogen, and formation of tyloses, could determine resistance and susceptibility, and could be conditioned by genetic and or physiologic causes.

Disease resistance is much commoner than disease susceptibility in that most plant species are resistant to most species of plant pathogens. On the other hand, disease resistance is a less stable property of plants than is disease susceptibility in that there are more examples where resistant plants have been predisposed toward susceptibility, than where susceptible plants have been predisposed toward resistance. There are many cases of successful artificial inoculation of plants with pathogens which have not been found associated with these plants in nature. This could be because pathogen and host did not come into effective contact in nature, or because field observations have not been sufficiently intensive, but predisposition probably plays a large part. Many studies of host ranges of pathogens and of disease susceptibility are performed in glasshouses, and growing plants in glasshouses certainly increases the susceptibility of plants to many diseases. It is true that most foliage diseases, except powdery mildews, do not naturally occur in greenhouses, but this is because most foliage fungi require free moisture for germination and penetration, and glasshouses by their nature protect plants from rain and dew. When such glasshouse-grown plants are inoculated and appropriately incubated in a moist chamber for a few hours, they are usually more heavily infected than are comparable plants grown out of doors.

Just what are the principal predisposing features of glasshouse culture may not be known but reduced light and higher temperature are certainly involved. Also important is the fact that glasshouse plants are usually more liberally watered, fertilized, and cared for than are plants outdoors. The succulence resulting from glasshouse culture provides a favorable substrate for many pathogens. While different species of pathogens differ enormously in most characters, they are mostly similar in that to be successful they must enter and move through their hosts by mechanical pressure. Any character such as succulence which reduces normal mechanical resistance to pathogens is likely to favor disease.

### E. Use in Further Studies

A "practical" use of predisposition in the future will certainly be in further studies of disease transmission, host range, epidemiology, etc. One of the elementary difficulties in studying disease is that of securing abundant artificial transmission. Predisposing treatments of the host may be helpful. For example, the symptoms of epidemic wildfire of tobacco, caused by *Pseudomonas tabaci*, could not be reproduced at will till Clayton (1936) demonstrated the important role of water soaking before inoculation in the development of this disease. Shading of plants before inoculation (Samuel and Bald, 1933; Bawden and Roberts, 1948), addition of phosphate to the inoculum (Thornberry, 1935; Yarwood, 1952b), and heating of plants before inoculation (Yarwood, 1956b) have been useful in the transmission of many viruses and are interpreted here as predisposition effects. There are still many viruses such as aster yellows virus and peach mosaic virus which have not been transmitted artificially, and it is hoped that predisposing treatments reported here and others as yet undiscovered will be useful.

When certain insects and bacteria are subjected to appropriate stimuli, viruses not previously demonstrable may become manifest and continue to be manifest in succeeding generations of the insects and bacteria long after the stimulus is removed. It is commonly believed that these insects and bacteria were carrying latent infections, and that the stimuli applied caused the infection to become active. It might be said that the stimuli disposed infected, but nondiseased, individuals to become diseased. No such phenomenon is recognized in higher plants but such cases are likely to be found in the future. Virus-infected plants may show symptoms in one environment but not in another, but in most of these cases the plants without symptoms will yield infective inoculum. Fruit trees may carry several viruses without showing symptoms. It seems likely that if appropriate stimuli were applied, specific symptoms would be manifested. When certain strains of peach ring spot virus, which are commonly latent in fruit trees, are transferred to beans, they normally cause no symptoms in the inoculated leaves, but if the inoculated leaves are heated at 50° C. for about 30 seconds for 2 to 11 days after inoculation, lesions may become apparent a few days later. This duration and temperature of heat treatment are approximately the same as those which are effective in predisposing beans to several viruses (Yarwood, 1956b). Therefore, in some cases at least the same heat treatment may increase the invasiveness of the pathogen if applied before or after infection. That this is not a general rule, however, is apparent from studies with the bean rust fungus, where 30 seconds at 45° C. before

inoculation increased infection of the heated leaves, whereas only slightly more time at 45° after infection was therapeutic.

The principal utility of predisposition in the near future will be as an aid in working with and understanding disease. Use of predisposition in disease control lies farther ahead.

## REFERENCES

- Ackley, W. B. 1954. Seasonal and diurnal changes in the water contents and water deficits of Bartlett pear leaves. *Plant Physiol.* **29**: 445-448.
- Adami, J. G. 1910. "The Principles of Pathology." Vol. 1. Lea & Febiger, Philadelphia, Pennsylvania.
- Ainsworth, G. C., and G. R. Bisby. 1943. A dictionary of the fungi. *Imp. Mycol. Inst. Kew.*
- Allen, J. D. 1957. The development of potato skin-spot disease. *Ann. Appl. Biol.* **45**: 293-298.
- Allington, W. B., and C. V. Feaster. 1946. The relation of stomatal behavior at the time of inoculation to the severity of infection of soybeans by *Xanthomonas phaseoli* var. *sojense* (Hedges) (Starr) Burk. *Phytopathology* **36**: 385-386.
- Alten, F., and H. Orth. 1941. Untersuchungen über den Aminosäuregehalt und die Anfälligkeit der Kartoffel gegen die Kraut- und Knollenfäule (*Phytophthora infestans* d. By.). *Phytopathol. Z.* **13**: 243-271.
- American Phytopathological Society. 1940. Report of the committee on technical words. *Phytopathology* **30**: 361-368.
- Anderson, H. W. 1956. "Diseases of Fruit Crops." McGraw-Hill, New York.
- Anderson, H. W., and D. Powell. 1950. Relation of time of day and other factors in mass inoculation with *Xanthomonas pruni*. *Phytopathology* **40**: 1.
- Arey, L. B., W. Burrows, J. P. Greenhill, and R. M. Underhill (eds.). 1957. "Dorlands Illustrated Medical Dictionary." Saunders, Philadelphia, Pennsylvania.
- Baker, K. F. 1946. Observations on some Botrytis diseases in California. *Plant Disease Rptr.* **30**: 145-155.
- Baltzer, U. 1930. Untersuchungen über die Anfälligkeit des Roggens für Fusariosen. *Phytopathol. Z.* **2**: 377-441.
- Bawden, F. C. 1950. "Plant Viruses and Virus Diseases." *Chronica Botanica*, Waltham, Massachusetts.
- Bawden, F. C., and F. M. Roberts. 1948. Photosynthesis and predisposition of plants to infection with certain viruses. *Ann. Appl. Biol.* **35**: 418-428.
- Bawden, F. C., and B. Kassanis. 1950. Some effects of host nutrition on the susceptibility of plants to infection by certain viruses. *Ann. Appl. Biol.* **37**: 46-57.
- Bennett, C. W., and A. S. Costa. 1949. Tristeza disease of citrus. *J. Agr. Research* **78**: 207-237.
- Berwith, C. E. 1936. Apple powdery mildew. *Phytopathology* **26**: 1071-1073.
- Bewley, W. F. 1923. "Diseases of Glasshouse Plants." Benn, London.
- Blodgett, E. C. 1936. The anthracnose of currant and gooseberry caused by *Pseudopeziza ribis*. *Phytopathology* **26**: 115-152.
- Blumer, S., L. Stalder, and A. Harder. 1956. Über die gegenseitigen Beziehungen zwischen Gurkenmosaik und Gurkenmehltau (Vorläufige Mitteilung). *Phytopathol. Z.* **25**: 39-54.
- Boning, K. 1930. Beiträge zur Kenntnis des parasitischen Verhaltens von *Pseudo-*



- monas tabaci* Wolf et Foster des Wildfeuer Erregers am Tabak. *Z. Parasitenk.* **2**: 645-755.
- Bortels, H. 1947. Festschrift zur Feier des achtzigsten Geburtstages von Geh.-Rat. Prof. h.c. Otto Appel. 68 p. *Biol. Zentralanst. Land- u. Forstwirts. in Berlin-Dahlem*.
- Boubals, D., A. Vergnes, and H. Bobo. 1955. Essais de fongicides organique dans la lutte contre le mildious de la Vigne effectuees en 1954. *Progr. agr. et vit.* **143**: 64-74.
- Boyd, A. E. W. 1952. Dry-rot disease of the potato. *Ann. Appl. Biol.* **39**: 330-338, 351-357.
- Boyd, A. E. W., and J. M. Henderson. 1953. Susceptibility of immature potato tubers to blight. *Plant Pathol.* **2**: 113-116.
- Boyd, A. E. W., and G. R. White. 1956. Effect of potash on the internal blackening of potatoes. *Plant Pathol.* **5**: 107-109.
- Braun, A. C. 1952. Conditioning of the host cell as a factor in the transformation process in crown gall. *Growth* **16**: 65-74.
- British Mycological Society. 1950. Definitions of some terms used in plant pathology. *Brit. Mycol. Soc. Trans.* **33**: 154-160.
- Bromfield, L. 1948. "Malabar Farm." Harper, New York.
- Brooks, F. T. 1908. Observations on the biology of *Botrytis cinerea*. *Ann. Botan.* **22**: 479-487.
- Brooks, F. T., and W. C. Moore. 1926. Silver leaf disease V. *J. Pomol. Hort. Sci.* **5**: 61-97.
- Brown, B. E. 1930. Note regarding a possible influence of soil reaction on development of powdery mildew of cowpeas. *Phytopathology* **20**: 683-685.
- Brown, W. 1936. The physiology of host-parasite relations. *Botan. Rev.* **2**: 236-281.
- Brown, W., and C. C. Harvey. 1927. Studies in the physiology of parasitism. X. *Ann. Botany (London)* **41**: 643-662.
- Browning, J. A. 1954. Breakdown of rust resistance in detached leaves of normally resistant oat varieties. *Phytopathology* **44**: 483.
- Burke, D. W., and C. E. Seliskar. 1957. Disease incidence and yields of beans in relation to cultivation injury in northeastern Colorado. *Plant Disease Repr.* **41**: 483-487.
- Butin, H. 1957. Untersuchungen über Resistenz und Krankheitsanfälligkeit der Pappel gegenüber *Dothichiza populea* Sacc. et Br. *Phytopathol. Z.* **28**: 353-374.
- Butler, E. J., and S. G. Jones. 1949. "Plant pathology." Macmillan, New York.
- Buxton, E. W. 1957. Some effects of pea root exudates on physiologic races of *Fusarium oxysporum* Fr. f. *pisi* (Linf.) Snyder and Hansen. *Brit. Mycol. Soc. Trans.* **40**: 145-154.
- Buxton, E. W., and F. T. Last. 1956. *Rept. Rothamsted Exptl. Sta.* **1955**.
- Caldwell, R. M., and G. M. Stone. 1936. Relation of stomatal function of wheat to invasion and infection by leaf rust (*Puccinia triticina*). *J. Agr. Research* **52**: 917-932.
- Caroselli, N. E., and A. W. Feldman. 1951. Dutch elm disease in young elm seedlings. *Phytopathology* **41**: 46-51.
- Carpenter, J. B. 1949. Production and discharge of basidiospores by *Pellicularia filamentosa* (Pat.) Rogers on Hevea rubber. *Phytopathology* **39**: 980-985.
- Castano, J. J., and M. F. Kernkamp. 1956. The influence of certain plant nutrients on infection of soybeans by *Rhizoctonia solani*. *Phytopathology* **46**: 326-328.

- Chapman, H. D., and S. M. Brown. 1942. Some fungal infections of citrus in relation to nutrition. *Soil Sci.* **54**: 303-312.
- Cheo, P. C., G. S. Pound, and L. G. Weathers. 1952. The relation of host nutrition to the concentration of cucumber virus 1 in spinach. *Phytopathology* **42**: 377-381.
- Cherewick, W. J. 1944. Studies on the biology of *Erysiphe graminis* DC. *Can. J. Research* **C22**: 52-86.
- Chester, K. S. 1946. "The Cereal Rusts." *Chronica Botanica*, Waltham, Massachusetts.
- Chester, K. S. 1947. "Nature and Prevention of Plant Diseases." Blakiston, Philadelphia, Pennsylvania.
- Chibnall, A. C. 1924. Diurnal variation in the protein nitrogen of runner bean leaves. *Biochem. J.* **18**: 387.
- Clayton, E. E. 1936. Water soaking of leaves in relation to development of the wildfire disease of tobacco. *J. Agr. Research* **52**: 239-269.
- Clayton, E. E. 1945. Resistance of tobacco to blue mold (*Peronospora tabacina*). *J. Agr. Research* **70**: 79-87.
- Coffman, F. A. 1957. Cold-resistant oat varieties also resistant to heat. *Science* **125**: 1298-1299.
- Cohen, M. 1951. Increased resistance to bean rust associated with water infiltration. *Phytopathology* **41**: 937.
- Colhoun, J. 1945. The effect of boron on the development of flax rust. *Gardeners' Chronicle* **118**: 191.
- Condit, I. J., and H. J. Stevens. 1919. "Die-back" of the fig in California. *Calif. Dept. Agr. Monthly Bull.* **8**: 61-63.
- Cornford, C. E. 1953. *Rept. Rothamsted Expt. Sta.* **1952**: 79-92.
- Cox, R. S. 1954. Effect of temperature on the development of downy mildew of lima bean. *Phytopathology* **44**: 325-327.
- Crafts, A. S., and H. G. Reiber. 1948. Herbicidal properties of oils. *Hilgardia* **18**: 77-156.
- Crowdy, S. H. 1952. Observations on apple canker. IV. The infection of leaf scars. *Ann. Appl. Biol.* **39**: 569-580.
- Crowdy, S. H., and R. L. Wain. 1950. Aryloxyaliphatic acids as systemic fungicides. *Nature* **165**: 937-938.
- Croxall, H. E., T. M. Norman, and D. C. Gwyne. 1957. Effect of 2:4:6 trichlorophenoxyacetic acid on the susceptibility of tomato plants to *Didymella lycopersici*. *Plant Pathol.* **6**: 27-31.
- Davis, D., and A. E. Dimond. 1952. Altering resistance to disease with synthetic organic chemicals. *Phytopathology* **42**: 563-578.
- Davis, D., and A. E. Dimond. 1953. Inducing disease resistance with plant growth-regulators. *Phytopathology* **43**: 137-140.
- Diachun, S., W. D. Valteau, and F. M. Johnson. 1944. Invasion of water-soaked tobacco leaves by bacteria, solutions, and tobacco mosaic virus. *Phytopathology* **34**: 250-253.
- Dickson, J. G. 1923. Influence of soil temperature and moisture on the development of seedling-blight of wheat and corn caused by *Gibberella saubinetii*. *J. Agr. Research* **23**: 837-870.
- Dillon Weston, W. A. R., and R. E. Taylor. 1944. Development of mold on the cut surfaces of potato tubers. *J. Agr. Sci.* **34**: 93-96.

- Dimond, A. E., and M. E. Corden. 1957. Reduction and promotion of the development of *Fusarium* wilt of tomato by gibberellic acid. *Phytopathology* **47**: 519.
- Dippenaar, B. J. 1933. Environmental and control studies of the common scab disease of potatoes caused by *Actinomyces scabies* (Thaxt.) Guss. *Union S. Africa Dept. Agr. Sci. Bull.* **136**: 1-78.
- Doak, K. D. 1954. Leaf rust reaction in relation to wheat fertilization in Indiana. *Better Crops with Plant Food* **38**: 18-22, 40-42.
- Donandt, S. 1932. Untersuchungen über die Pathogenität des Wirtelpilzes *Verticillium albo atrum* R. u. B. *Z. Parasitenk.* **4**: 653-711.
- Doran, W. L. 1932. Downy mildew of cucumbers. *Mass. Agr. Expt. Sta. Bull.* **283**: 1-22.
- Eaton, F. M. 1930. The effect of boron on powdery mildew and spot blotch of barley. *Phytopathology* **20**: 967-972.
- Edgerton, C. W., I. L. Forbes, P. J. Mills, J. Dufrenoy, and W. J. Luke. 1942. The hot water treatment of sugarcane. *Louisiana Agr. Expt. Sta. Bull.* **336**: 1-27.
- Edmundson, W. C. 1939. Sun injury to potato seed. *Am. Potato J.* **16**: 98-103.
- Erwin, A. T. 1921. Controlling downy mildew of lettuce. *Iowa Agr. Expt. Sta. Bull.* **196**: 305-328.
- Fawcett, H. S. 1936. "Citrus Diseases and Their Control." McGraw-Hill, New York.
- Fehmi, S. 1953. Beiträge zur Kenntnis der Wechselbeziehungen zwischen Kulturpflanzen, ihren Parasiten und der Umwelt. *Phytopathol. Z.* **6**: 543-588.
- Felton, M. W., and J. C. Walker. 1946. Environmental factors affecting downy mildew of cabbage. *J. Agr. Research* **72**: 69-81.
- Fischer, G. W., and C. S. Holton. 1957. "Biology and Control of the Smut Fungi." Ronald Press, New York.
- Fitzpatrick, R. E. 1935. Further studies on the parasitism of *Taphrina deformans*. *Sci. Agr.* **15**: 341-344.
- Fogg, G. E. 1944. Diurnal fluctuation in a physical property of leaf cuticle. *Nature* **154**: 515.
- Forsyth, F. R. 1957a. Effect of ions of certain metals on the development of stem rust in the wheat plant. *Nature* **179**: 217-218.
- Forsyth, F. R. 1957b. Studies on the physiology of resistance to cereal rusts. Summaries of papers. *IVth Intern. Congr. Crop Protect., Hamburg*, p. 13.
- Forward, D. F. 1957. Unpublished work. Cited by Forsyth (1957a).
- Foster, R. E., and J. C. Walker. 1947. Predisposition of tomato to *Fusarium* wilt. *J. Agr. Research* **74**: 165-185.
- Fred, E. B., I. L. Baldwin, and E. McCoy. 1932. "Root Nodule Bacteria and Leguminous Plants." Univ. Wisconsin, Madison, Wisconsin.
- Gallegly, M. E., Jr., and J. C. Walker. 1949. Plant nutrition in relation to disease development. *V. Am. J. Botany* **36**: 613-623.
- Gassner, G., and K. Hassebrauk. 1931. Untersuchungen über die Beziehungen zwischen Mineralsalznährung und Verhalten der Getreidepflanzen gegen Rost. *Phytopathol. Z.* **3**: 535-617.
- Gassner, G., and K. Hassebrauk. 1938. Untersuchungen über den Einfluss von Äther- und Chloroformnarkose auf das Rostverhalten junger Getreidepflanzen. *Phytopathol. Z.* **11**: 47-97.
- Gäumann, E. A. 1950. "Principles of Plant Infection" (English trans. by W. B. Brierley). Crosby Lockwood, London.
- Giddings, N. J. 1918. Infection and immunity in apple rust. *West V. Univ. Agr. Expt. Sta. Bull.* **170**: 1-71.
- Gothoskar, S. S., R. P. Scheffer, M. A. Stahmann, and J. C. Walker. 1955. Further

- studies on the nature of *Fusarium* resistance in tomato. *Phytopathology* **45**: 303-307.
- Grainger, J. 1956. Host nutrition and attack by fungal parasites. *Phytopathology* **46**: 445-456.
- Gregg, M. 1952. Studies in the physiology of parasitism. XVII. *Ann. Botany (London)* **16**: 235-250.
- Grossenbacher, K. A. 1938. Diurnal fluctuation in root pressure. *Plant Physiol.* **13**: 669-676.
- Hacker, R. G., and J. R. Vaughn. 1957. Cycloheximide analogues cause preinfection resistance to *Puccinia graminis* var. *tritici* in spring wheat. *Phytopathology* **47**: 14.
- Hartig, R. 1894. "Textbook of the Diseases of Trees." Country Life, London.
- Hassebrauk, K. 1940. Untersuchungen über den Einfluss einiger Aussenfaktoren auf das Anfälligkeitsverhalten der Standardsorten gegenüber verschiedenen physiologischen Rassen des Weizenbraunrostes. *Phytopathol. Z.* **12**: 233-276.
- Hatfield, W. C., J. C. Walker, and J. H. Owen. 1948. Antibiotic substances in onion in relation to disease resistance. *J. Agr. Research* **77**: 115-135.
- Heald, F. D. 1933. "Manual of Plant Diseases." McGraw-Hill, New York.
- Heggeness, H. G. 1942. Effect of borax applications on the incidence of rust on flax. *Plant Physiol.* **17**: 143-144.
- Hewitt, W. B. 1938. Leaf-scar infection in relation to the olive-knot disease. *Hilgardia* **12**: 41-71.
- Hiley, W. E. 1919. "The Fungal Diseases of the Common Larch." Oxford Univ. Press, London and New York.
- Hirst, J. M. 1953. Changes in the atmospheric spore content: diurnal periodicity and the effects of weather. *Brit. Mycol. Soc. Trans.* **36**: 375-393.
- Hitchborn, J. H. 1954. Studies in the susceptibility of plants to viruses. Ph.D. Thesis. Cambridge Univ., Cambridge.
- Hoerr, N. L., and A. Osol (eds.). 1956. "Blakistons New Gould Medical Dictionary." McGraw-Hill, New York.
- Holmes, F. O. 1929. Inoculating methods in tobacco mosaic studies. *Botan. Gaz.* **87**: 56-63.
- Holton, C. S., and F. D. Heald. 1936. Studies on the control and other aspects of bunt of wheat. *Wash. State Coll. Agr. Expt. St. Bull.* **339**: 1-35.
- Hopkins, E. F., and K. W. Loucks. 1946. Effect of heavy metals on susceptibility of oranges to stem-end rots. *Am. J. Botany* **33**: 837.
- Horsfall, J. G. 1945. "Fungicides and Their Action." *Chronica Botanica*, Waltham, Massachusetts.
- Horsfall, J. G. 1956. "Principles of Fungicidal Action." *Chronica Botanica*, Waltham, Massachusetts.
- Horsfall, J. G., and A. E. Dimond. 1957. Interactions of tissue sugar, growth substances, and disease susceptibility. *Z. Pflanzenkrankh u. Pflanzenschutz* **64**: 415-421.
- Howard, Sir A. 1940. "An Agricultural Testament." Oxford Univ. Press, London and New York.
- Hubert, E. E. 1931. "An Outline of Forest Pathology." Wiley, New York.
- Ibrahim, I. A. 1951. Effect of 2,4-D on stem-rust development in oats. *Phytopathology* **41**: 951-953.
- Isleib, D. R. 1957. The effect of gamma irradiation on suberization and periderm formation in potato. *Am. Potato J.* **34**: 76.
- Ismailov, K. A. 1954. (Micro-elements and the increase of resistance in wheat to



- yellow rust.) *Proc. Acad. Sci. Azerbaijan S.S.R.* **10**: 491-494 (Abstr. in *Rev. Appl. Mycol.* **35**: 759, 1956).
- Iwata, Y. 1951. Studies on the relations between leaf maturity of cucumber and infection by downy mildew. *Mie Daigaku Nôgakubu Gakujutsu Hôkoku* **2**: 34-42.
- Jedlinski, H. 1956. Plant virus infection in relation to the interval between wounding and inoculation. *Phytopathology* **46**: 673-676.
- Johnson, J. 1937. Relation of water-soaked tissues to infection by *Bacterium angulatum* and *Bact. tabacum*. *J. Agr. Research* **55**: 599-618.
- Johnson, J. 1947. Water congestion and fungus parasitism. *Phytopathology* **37**: 403-417.
- Johnson, T. 1946. The effect of DDT on the stem rust reaction of Khapli wheat. *Can. J. Research* **C24**: 23-25.
- Kassanis, B. 1952. Some effects of high temperature on the susceptibility of plants to infection with viruses. *Ann. Appl. Biol.* **39**: 358-369.
- Kassanis, B. 1957. Effects of changing temperature on plant virus diseases. *Advances in Virus Research* **4**: 221-241.
- Kendrick, J. B., Jr., and J. C. Walker. 1948. Predisposition of tomato to bacterial canker. *J. Agr. Research* **77**: 169-186.
- Kendrick, J. B., Jr., J. T. Middleton, and E. F. Darley. 1954. Chemical protection of plants from ozonated olefin (smog) injury. *Phytopathology* **44**: 494-495.
- Kent, N. L. 1941. The influence of lithium salts on certain cultivated plants and their parasitic diseases. *Ann. Appl. Biol.* **28**: 189-209.
- Kerling, L. C. P. 1952. (Damage and fungal attack on peas as consequences of night frost.) *Tijdschr. Plantenziekten* **58**: 29-54 (Abstr. in *Rev. Appl. Mycol.* **32**: 163, 1953).
- Kernkamp, M. F., D. J. de Zeeuw, S. M. Chen, B. C. Ortega, C. T. Tsiang, and A. M. Khan. 1952. Investigations on physiologic specialization and parasitism of *Rhizoctonia solani*. *Univ. Minn. Agr. Expt. Sta. Tech. Bull.* **200**: 1-36.
- Keyworth, W. G., and A. E. Dimond. 1952. Root injury as a factor in the assessment of chemotherapeutants. *Phytopathology* **42**: 311-315.
- Koritz, H. G., and F. W. Went. 1953. The physiological action of smog on plants. I. *Plant Physiol.* **28**: 50-62.
- Krotkov, G. 1943. Diurnal changes in the carbohydrates of wheat leaves. *Can. J. Research* **C21**: 26-40.
- Kunkel, L. O. 1934. Studies on acquired immunity with tobacco and aucuba mosaics. *Phytopathology* **24**: 437-466.
- Lambertz, P. 1954. Untersuchung über das Vorkommen von Plasmodiesmen in den Epidermisaussenwänden. *Planta* **44**: 147-190.
- Larner, F. G. 1937. Keeping quality of sugar beets as influenced by growth and nutritional factors. *J. Agr. Research* **54**: 185-198.
- Lasser, E. 1938. Der Einfluss von Licht und Jarowization auf den Befall von Weizen, Hafer und Gerste durch *Tilletia*, *Ustilago*, und *Helminthosporium*. *Kuhn-Arch.* **44**: 161-210.
- Laude, H. H. 1939. Diurnal cycle of heat resistance in plants. *Science* **89**: 556-557.
- Lauritzen, J. I., and R. C. Wright. 1934. Factors affecting gladiolus in storage. *J. Agr. Research* **48**: 265-282.
- Leach, J. C. 1929. The effect of grafting on resistance and susceptibility of beans to *Colletotrichum lindemuthianum*. *Phytopathology* **19**: 875-877.

- Leach, L. D., and A. E. Davey. 1942. Reducing southern *Sclerotium* rot of sugar beets with nitrogenous fertilizers. *J. Agr. Research* **64**: 1-18.
- Leben, C., and R. W. Fulton. 1951. The inhibition of virus symptom expression by sodium azide, potassium cyanide, and two antibiotics. *Phytopathology* **41**: 23.
- Levitt, J. 1941. "Frost Killing and Hardiness of Plants." Burgess, Minneapolis, Minnesota.
- Longchamp, R., M. Roy, and R. Gautheret. 1951. Action de l'ester éthylique du 2,4-D sur le développement de *Claviceps purpurea* dans le champs de blé. *Compt. rend.* **233**: 888-890.
- Loughnane, J. B., R. McKay, and H. A. Lafferty. 1946. Observations on the Pasmio disease of flax and on the causal fungus *Sphaerella linorum* Wollenweber. *Sci. Proc. Roy. Dublin Soc.* **24**: 89-98.
- Lowig, E. 1936. Weitere Versuche zur Frage der Abhängigkeit der Mehlauresistenz von der Ernährung der Pflanze. *Ernähr. Pflanze* **32**: 61-67.
- Lyles, W. E., M. C. Futrell, and I. M. Atkins. 1957. The effect of plant growth regulators on the response of wheat varieties to leaf rust. *Proc. Stanford Meeting Am. Soc. Plant Physiol.* **1957**: xliii.
- McWhorter, F. P. 1945. The diseases of *Lilium longiflorum* in the Pacific Northwest. *Plant Diseases Repr.* **29**: 40-44.
- Martin, W. J., L. D. Newsom, and J. E. Jones. 1956. Relationship of nematodes to the development of *Fusarium* wilt in cotton. *Phytopathology* **46**: 285-289.
- Mason, T. G., and E. J. Maskell. 1928. A study of diurnal variation in the carbohydrates of leaf, bark, and wood, and of the effects of ringing. *Ann. Botany (London)* **42**: 189-253.
- Matthews, R. E. F. 1953. Factors affecting the production of local lesions by plant viruses. I. The effect of time of day of inoculation. *Ann. Appl. Biol.* **40**: 377-383.
- Maximov, N. A. 1930. "A Textbook of Plant Physiology," 381 pp. McGraw-Hill, New York.
- Melander, L. W., and J. H. Craigie. 1927. Nature of resistance of *Berberis* spp. to *Puccinia graminis*. *Phytopathology* **17**: 95-114.
- Miller, E. C. 1938. "Plant Physiology." McGraw-Hill, New York.
- Minkevičius, A. 1932. Untersuchungen über den Einfluss der Narkose auf die Pilzempfindlichkeit der Pflanzen. *Phytopathol. Z.* **5**: 99-152.
- Mishra, J. N. 1953. Resistance of potato tubers to certain parasitic fungi. *Phytopathology* **43**: 338-340.
- Mix, A. J. 1930. Brown-rot leaf and twig blight following peach-leaf curl. *Phytopathology* **20**: 265-266.
- Moore, W. C. 1944. Chocolate spot of beans. *Agriculture (Engl.)* **51**: 266-269.
- Moshkov, B. S. 1938. (Photoperiodism and immunity.) *Compt. rend. acad. sci. U.R.S.S.* **19**: 751-754 (Abstr. in *Rev. Appl. Mycol.* **18**: 39, 1939).
- Mostafa, M. A., and S. K. Gayed. 1956. Effect of herbicide 2,4-D on bean chocolate-spot disease. *Nature* **178**: 502.
- Müller, K. O., and J. Monro. 1951. The reaction of virus-infected potato plants to *Phytophthora infestans*. *Ann. Appl. Biol.* **38**: 765-773.
- Murant, A. F., and R. K. S. Wood. 1957. Factors affecting the pathogenicity of bacteria to potato tubers. II. *Ann. Appl. Biol.* **45**: 650-663.
- Murray, G. 1880. The spread of the potato disease. *Gardners' Chronicle* **14**: 784.

- Natti, J. J., G. E. R. Hervey, and C. B. Sayre. 1956. Factors contributing to the increase of downy mildew of broccoli in New York State and its control with fungicides and agrimycin. *Plant Disease Repr.* **40**: 118-124.
- Naylor, A. W. 1951. Accumulation of sucrose in maize following treatment with maleic hydrazide. *Arch. Biochem. Biophys.* **33**: 340-342.
- Neal, D. C. 1954. The reniform nematode and its relationship to the incidence of *Fusarium* wilt of cotton at Baton Rouge, Louisiana. *Phytopathology* **44**: 447-450.
- Nelson, K. E. 1951. Factors influencing the infection of table grapes by *Botrytis cinerea* (Pers.) *Phytopathology* **41**: 319-326.
- Newhall, A. G. 1944. A serious storage rot of celery caused by the fungus *Anisotropa macrospora* n. gen. *Phytopathology* **34**: 92-105.
- Newton, W. 1952. Effects of application of fungicides to wounded plant tissues. *Sci. Agr.* **32**: 659-662.
- Panzer, J. D. 1957a. Osmotic pressure and plant virus local lesions. *Phytopathology* **47**: 337-341.
- Panzer, J. D. 1957b. Mineral salts as related to plant virus local lesions. *Phytopathology* **47**: 453.
- Pasteur, L., J. Joubert, and C. Chamberlain. 1878. La théorie des germes et ses applications à la médecine et à la chirurgie. *Compt. rend.* **86**: 1037-1043.
- Patel, J. S., and A. P. B. Nayar. 1936. Natural and induced resistance to shoot rot in the coconut. *Proc. Indian Acad. Sci.* **3**: 432-437.
- Phillis, F., and T. G. Mason. 1942. On diurnal variations in the mineral content of the cotton plant. *Ann. Botany (London)* **6**: 437-442.
- Pitcher, R. S., and P. C. R. Webb. 1949. A fungus disease of raspberries induced by insect attack. *Nature* **163**: 574-575.
- Pound, G. S., and L. G. Weathers. 1953. The relation of host nutrition to multiplication of turnip virus 1 in *Nicotiana glutinosa* and in *N. multivalvis*. *Phytopathology* **43**: 669-674.
- Pucher, G. W., C. S. Leavenworth, W. D. Ginter, and H. B. Vickery. 1947. The diurnal variation in organic acid and starch content of *Bryophyllum calycinum*. *Plant Physiol.* **22**: 360-376.
- Raines, M. A. 1922. Vegetative vigor of the host as a factor influencing susceptibility and resistance to certain rust diseases of the higher plants. *Am. J. Botany* **9**: 183-203, 215-238.
- Rawlins, T. E., and K. C. Parker. 1934. Influence of rootstocks on the susceptibility of sweet cherry to the buckskin disease. *Phytopathology* **24**: 1029-1031.
- Riker, A. J. 1929. Studies on the influence of environment on infection by certain bacterial plant parasites. *Phytopathology* **19**: 96.
- Riviera, V. 1924. (Cryptogamic epidemics and the environmental factors that determine them.) *Intern. Rev. Sci. Pract. Agr.* **2**: 604-609 (Abstr. in *Rev. Appl. Mycol.* **4**: 108, 1925).
- Roberts, F. M. 1944. Factors influencing infection of the tomato by *Verticillium albo-atrum*. II. *Ann. Appl. Biol.* **31**: 191-193.
- Roberts, J. W., and J. C. Dunegan. 1932. Peach brown rot. *U. S. Dept. Agr. Tech. Bull.* **328**.
- Roemer, T., W. H. Fuchs, and K. Isenbeck. 1938. Die Züchtung resistenter Rassen der Kulturpflanzen. *Kuhn-Arch.* **45**: 427.
- Rose, D. H., C. O. Bratley, and W. T. Penzer. 1939. Market diseases of fruits and vegetables: grapes and other small fruits. *U. S. Dept. Agr. Misc. Publ.* **340**: 1-26.

- Ross, A. F. 1953. *Physalis floridana* as a local lesion test plant for potato virus Y. *Phytopathology* **43**: 1-8.
- Rowell, J. B. 1953. Leaf blight of tomato and potato plants. *Rhode Island Univ. Agr. Expt. Sta. Bull.* **320**: 1-29.
- Salmon, E. S. 1905. Further cultural experiments with biologic forms of the Erysiphaceae. *Ann. Botany* **19**: 125-148.
- Samborski, D. J., and M. Shaw. 1957. Unpublished work. Cited by Forsyth (1957a).
- Samuel, G., and J. G. Bald. 1933. On the use of primary lesions in quantitative work with two plant viruses. *Ann. Appl. Biol.* **20**: 70-99.
- Sanford, G. B. 1951. Effect of various chemicals on the natural healing of freshly cut potato sets. *Phytopathology* **41**: 1077-1082.
- Schaffnit, E. 1922. Zur Bekämpfung der Pilzkrankheiten des Getreidekorns. *Landwirtsch. Jahrb.* **57**: 259-283.
- Schaffnit, E., and K. Meyer-Hermann. 1930. Ueber den Einfluss der Bodenreaktion auf die Lebensweise von Pilzparasiten und das Verhalten ihrer Wirtspflanzen. *Phytopathol. Z.* **2**: 99-166.
- Scheffer, R. P. 1957. Analysis of *Fusarium* resistance in tomato by grafting experiments. *Phytopathology* **47**: 328-331.
- Schmitt, C. 1952. Influence de la lumière sur la résistance de plantules de *Lepidium sativum* L. à *Botrytis cinerea* Pers. *Compt. rend.* **235**: 258-260.
- Schnathorst, W. C., and A. R. Weinhold. 1957. An osmotic mechanism for resistance to powdery mildew in lettuce and peach. *Phytopathology* **47**: 533.
- Schuldt, P. H. 1955. Comparison of anthracnose fungi on oak, sycamore, and other trees. *Contribs. Boyce Thompson Inst.* **18**: 85-107.
- Schuster, C. E., and R. E. Stephenson. 1940. Sunflower as an indicator plant of boron deficiency in soils. *J. Am. Soc. Agron.* **32**: 607-621.
- Schwinghamer, E. A. 1957. Effect of ionizing radiation on rust reaction in plants. *Science* **125**: 23-24.
- Sempio, C. 1938. (On an experimental case of distinct antagonism *in vivo* (*Tilletia caries*)—*Erysiphe graminis* on "Mentana")). *Riv. patol. vegetale* **28**: p. 377-384. (Abstr. in *Rev. Appl. Mycol.* **18**: 166, 1939).
- Shaw, L. 1935. Intercellular humidity in relation to fire-blight susceptibility in apple and pear. *Cornell Univ. Agr. Expt. Sta. Mem.* **181**: 1-40.
- Sironval, C. 1951. Un exemple de lutte physiologique contre l'infection. *Lejeunia* **15**: 51-54 (Abstr. in *Rev. Appl. Mycol.* **34**: 455, 1955).
- Sitterly, W. R., E. B. Williams, and J. R. Shay. 1957. Factors influencing infection of apple fruits by *Botryosphaeria ribis*. *Phytopathology* **47**: 32.
- Smith, R. E. 1931. The life history of *Sclerotinia sclerotiorum* with reference to the green rot of apricots. *Phytopathology* **21**: 407-423.
- Sorauer, P. 1880. Gibt es eine Prädisposition der Pflanzen für Krankheiten? *Landwirtsch. Vers. Sta.* **25**: 327-372.
- Spencer, E. L. 1935. Effect of nitrogen supply on host susceptibility to virus infection. *Phytopathology* **25**: 178-191.
- Spinks, G. T. 1913. Factors affecting susceptibility to disease in plants. *J. Agr. Sci.* **5**: 231-247.
- Stakman, E. C. 1914. A study in cereal rusts: physiological races. *Minn. Univ. Agr. Expt. Sta. Bull.* **138**: 1-56.
- Straib, W., and A. Noll. 1944. Untersuchungen über den Einfluss der Hitze auf den Rostparasitismus. *Zentr. Bakteriöl. Parasitenk., Abt. II.* **106**: 257-277.
- Suchorukov, K. T. 1957. The physiology of immunity of some agricultural plants. *Proc. 2nd Intern. Conf. Plant Protect., Fernhurst Research Sta., Engl.* pp. 42-52.



- Tapke, V. F. 1951. Influence of preinoculation environment on the infection of barley and wheat by powdery mildew. *Phytopathology* **41**: 622-632.
- Thomas, H. E. 1921. The relation of the health of the host and other factors to infection of *Apium graveolens* by *Septoria apii*. *Bull. Torrey Botan. Club* **48**: 1-29.
- Thomas, H. E., and P. A. Ark. 1934. Nectar and rain in relation to fire blight. *Phytopathology* **24**: 682-685.
- Thornberry, H. H. 1935. Effect of phosphate buffers on infectivity of tobacco-mosaic virus. *Phytopathology* **25**: 618-627.
- Thorold, C. A. 1955. Observations on black-pod disease (*Phytophthora palmivora*) of cacao in Nigeria. *Brit. Mycol. Soc. Trans.* **38**: 435-452.
- Tinsley, T. W. 1953. The effects of varying the water supply of plants on their susceptibility to infection with viruses. *Ann. Appl. Biol.* **40**: 750-760.
- Townsend, G. R. 1939. Diseases of beans in southern Florida. *Florida Univ. Agr. Expt. Sta. Bull.* **336**: 1-60.
- Trelease, S. F., and H. M. Trelease. 1928. Susceptibility of wheat to mildew as influenced by salt nutrition. *Bull. Torrey Botan. Club* **55**: 41-68.
- Valleau, W. D., E. N. Fergus, and L. Henson. 1933. Resistance of red clovers to *Sclerotinia trifoliorum* Erik., and infection studies. *Kentucky Agr. Expt. Sta. Research Bull.* **341**: 113-131.
- Van Arsdel, E. P., A. J. Riker, and R. F. Patton. 1956. The effects of temperature and moisture on the spread of white pine blister rust. *Phytopathology* **46**: 307-318.
- Vasudeva, R. S. 1930. Studies in the physiology of parasitism. XI. *Ann. Botany (London)* **44**: 469-493.
- Vohl, G. J. 1938. Untersuchungen über den Braunrost des Weizens *Puccinia tritici* Erikss. *Z. Pflanzenzücht.* **22**: 233-270.
- Volk, A. 1931. Einflüsse des Bodens, der Luft und des Lichtes auf die Empfänglichkeit der Pflanzen für Krankheiten. *Phytopathol. Z.* **3**: 1-88.
- Wade, G. C. 1956. Investigations on brown rot of apricots caused by *Sclerotinia fructicola* (Wint.) Rehm. *Australian J. Agr. Research* **7**: 504-526.
- Wager, V. A. 1945. Compost and disease. *Proc. 19th Ann. Congr. S. African Sugar Technologists' Assoc.* pp. 85-90.
- Waggoner, P. E., and A. E. Dimond. 1956. Altering disease resistance with ionizing radiation. *Phytopathology* **46**: 125-127.
- Wagner, F. 1940. Die Bedeutung der Kieselsäure für das Wachstum einiger Kulturpflanzen, ihren Nährstoffhaushalt und ihre Anfälligkeit gegen echte Mehltaupilze. *Phytopathol. Z.* **12**: 427-479.
- Walker, J. C. 1950. "Plant Pathology." McGraw-Hill, New York.
- Walker, J. C., M. E. Callegly, Jr., J. R. Bloom, and R. P. Scheffer. 1954. *Verticillium* wilt of tomato. *Am. J. Botany* **41**: 760-762.
- Ward, H. M. 1890. On some relations between host and parasite in certain epidemic diseases of plants. *Proc. Roy. Soc.* **47**: 393-443.
- Ward, H. M. 1901. "Diseases in Plants." Macmillan, London.
- Ward, H. M. 1902. On predisposition and immunity. *Proc. Cambridge Phil. Soc.* **11**: 307-328.
- Ward, H. M. 1905. Recent researches on the parasitism of fungi. *Ann. Botany (London)* **19**: 1-54.
- Watson, D. J. 1953. Botany Department. *Rept. Rothamsted Expt. Sta.* **1952**: 65-73 (Abstr. in *Rev. Appl. Mycol.* **33**: 653, 1954).

- Weatherley, P. E. 1951. Diurnal and seasonal variations in relative turgidity and environmental factors. *New Phytologist* **50**: 36-51.
- Wei, C. T. 1937. Rust resistance in the garden bean. *Phytopathology* **27**: 1090-1105.
- Weiss, F., J. I. Lauritzen, and P. Brierley. 1928. Factors in the inception and development of *Fusarium* rot in stored potatoes. *U. S. Dept. Agr. Tech. Bull.* **62**: 1-58.
- Werner, H. O. 1938. Wound healing in potatoes (Triumph variety) as influenced by type of injury, nature of initial exposure, and storage conditions. *Nebraska Univ. Agr. Expt. Sta. Research Bull.* **102**: 1-40.
- Weston, W. H., Jr. 1924. Nocturnal production of conidia by *Sclerospora graminicola*. *J. Agr. Research* **27**: 771-784.
- Whetzel, H. H. 1918. "History of Phytopathology." Saunders, Philadelphia, Pennsylvania.
- Whetzel, H. H., L. R. Hesler, C. T. Gregory, and W. H. Rankin. 1925. "Laboratory Outlines in Plant Pathology." Saunders, Philadelphia, Pennsylvania.
- Wilhelm, A. F. 1944. Untersuchungen zur Frage einer chemischen Bekämpfung der Traubenfäule (*Botrytis cinerea*). *Wein u. Rebe* **26**: 29-49, 67-73.
- Wilson, A. R. 1937. The chocolate spot disease of beans (*Vicia faba* L.) caused by *Botrytis cinerea* Pers. *Ann. Appl. Biol.* **24**: 258-288.
- Wiltshire, G. H. 1957. The susceptibility of French beans to infection with tobacco necrosis virus. *Rept. Rothamsted Exptl. Sta.* **1956**: 95-96.
- Wolf, F. A. 1957. "Tobacco Diseases and Decays." Duke Univ. Press, Durham, North Carolina.
- Yarwood, C. E. 1934. The comparative behavior of four clover-leaf parasites on excised leaves. *Phytopathology* **24**: 797-806.
- Yarwood, C. E. 1936. The diurnal cycle of the powdery mildew *Erysiphe polygoni*. *J. Agr. Research* **52**: 645-657.
- Yarwood, C. E. 1937. The relation of light to the diurnal cycle of sporulation of certain downy mildews. *J. Agr. Research* **54**: 365-373.
- Yarwood, C. E. 1938. The effect of boron nutrition on the susceptibility of some plants to powdery mildews. *Phytopathology* **28**: 22.
- Yarwood, C. E. 1939. Powdery mildews of peach and rose. *Phytopathology* **29**: 282-284.
- Yarwood, C. E. 1941. Diurnal cycle of ascus maturation of *Taphrina deformans*. *Am. J. Botany* **28**: 355-357.
- Yarwood, C. E. 1946. Detached leaf culture. *Botany Rev.* **12**: 1-56.
- Yarwood, C. E. 1948. Apricot jacket rot. *Phytopathology* **38**: 919-920.
- Yarwood, C. E. 1949. Effect of soil moisture and mineral nutrient concentration on the development of bean powdery mildew. *Phytopathology* **39**: 780-788.
- Yarwood, C. E. 1951a. Associations of rust and virus infections. *Science* **114**: 127-128.
- Yarwood, C. E. 1951b. Hop cankers. *Plant Disease Reprtr.* **35**: 361-363.
- Yarwood, C. E. 1952a. Some relations of carbohydrate level of the host to plant virus infections. *Am. J. Botany* **39**: 119-124.
- Yarwood, C. E. 1952b. The phosphate effect in plant virus inoculations. *Phytopathology* **42**: 137-143.
- Yarwood, C. E. 1953. Pressure effects in fungus and virus infections. *Phytopathology* **43**: 70-72.

- Yarwood, C. E. 1954a. Mechanism of acquired immunity to a plant rust. *Proc. Natl. Acad. Sci. U. S.* **40**: 374-377.
- Yarwood, C. E. 1954b. Zinc increases susceptibility of bean leaves to tobacco mosaic virus. *Phytopathology* **44**: 230-233.
- Yarwood, C. E. 1955. Deleterious effects of water in plant virus inoculations. *Virology* **1**: 268-285.
- Yarwood, C. E. 1956a. Further aids in virus inoculations. *Phytopathology* **46**: 32.
- Yarwood, C. E. 1956b. Heat-induced susceptibility of beans to some viruses and fungi. *Phytopathology* **46**: 523-525.
- Yarwood, C. E. 1957. Powdery mildews. *Botan. Rev.* **23**: 235-300.
- Yarwood, C. E. 1958. Unpublished data.
- Yarwood, C. E., and J. T. Middleton. 1954. Smog injury and rust infection. *Plant Physiol.* **29**: 393-395.
- Young, R. A., and I. W. Deep. 1956. Increase in incidence of crown gall on Mazzard cherry following preplanting treatments with organic fungicides. *Phytopathology* **46**: 640.
- Zentmyer, G. A., J. G. Horsfall, and P. P. Wallace. 1946. Dutch elm disease and its chemotherapy. *Conn. Agr. Expt. Sta. (New Haven) Bull.* **498**: 1-70.

## CHAPTER 15

# Therapy

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## I. INTRODUCTION

Here in Volume I we shall discuss the physical and chemical therapy of the diseased plant; the treatment of the diseased plant as an individual, not the plant as a member of a population. We shall consider the plant as a "patient" not as a public health charge.



Protection is aimed at the healthy plant. Therapy is aimed at the diseased plant. Protection is aimed at the pathogen as it lives and moves between hosts. Therapy is aimed at the pathogen after it has arrived in and has established housekeeping in the host. Therapy is the cure of a sick plant, the mitigation of its symptoms, or the repair of the damage whether the pathogen be animate or inanimate.

The practicing plant doctor is interminably being presented with sick plants, singly or *en masse*. He usually gives the disease a name on the basis of symptoms, identifies the fungus on the basis of its structures present, and suggests a preventive measure. The grower too often still has a diseased plant and a confused mind, with the one clear, happy thought that he paid no fee—at least directly.

In the literature written for the advice of growers 50 years ago, "cure" of plant diseases meant to "get ahead of the enemy." The reader was told that internal fungi, when once they are established, must be treated by removal and burning of diseased parts. This is common advice today, though mortal for the suffering plant. As our knowledge of the biochemical nature and mechanisms of disease increases, there is more concern that the plant patient lives. The next decade or two should see a "break-through" in the therapy of the diseased plant.

This phase of plant pathology is still in the adolescent stage. The framework is outlined, but the ideas and applications that are necessary to reach full maturity are appearing here and there in the writings of many men. An attempt has been made to encompass and clarify today's concepts on plant therapy with the hope that progress can advance faster in this important field.

A plant is today thought of as a dynamic entity maintained in health by a correlated series of interdependent metabolic processes. Animate or inanimate factors may disturb the usual functional pattern and result in developmental changes. Therapy removes the cause of disease so that the normal mechanism of living can run smoothly.

Combating the causal agent after it has injured the plant is a form of disease control labeled by some as "disinfection." However, the concept should include not only the neutralizing or stopping of the injurious stimulus, but also the repair of the cellular damage done. Hence, the subject matter concerns the pathogen-host interactions during the time interval between "infection" and death of the plant or its organs—a life-saving action. Knowledge of the pathogen-host relationship must answer many questions before effective therapy can be carried out. Usually the extensiveness of the infection is first thought of in order to make use of physical means of therapy aimed at confining the zone of injury.

## II. PHYSICAL THERAPY

Two courses are open for the therapy of plant diseases, physical therapy and chemotherapy. In the former, the disease is fought with physical means, such as surgery, temperature modification, radiation, and moisture modification. In the latter, the disease is fought with chemicals that act topically or systemically.

### A. Surgery

Surgery is the removal of infected tissues to prevent additional damage. Sometimes the plants themselves are able to stop further injury by shedding diseased structures. The "shot hole" symptom in leaves of cherry and poplar is familiar to pathologists. Similarly, leaf cast may avoid infection of the twigs, petal fall may avoid involvement of the fruit, and fruit drop the involvement of the spur or stem. Self-therapy is accomplished by the shedding of infected bark where the growth of suberized tissues exceeds the rate of penetration of the pathogen through the cortex. While expressed physically, the underlying mechanism is biochemical.

An unusual therapeutic measure has been proposed for the American leaf spot ("Ojo de gallo") of coffee by Wellman (1950). Gemmae of *Mycena (Omphalia) citricolor* serve as the inoculum and are too heavy to be borne by air currents, requiring dissemination by splashing rain. Hence, complete defoliation of the diseased trees rids them of the fungus.

A chemical "pruning" may be effective for ridding tomato plants of the root knot nematode. While evaluating the nematocidal activity of chemicals, Hopper and Tarjan (1954) observed that following chemical treatment of the soil with *p*-chlorophenyl rhodanine around young infected tomato plants they grew normally. Careful examination revealed that the infected roots were killed, but the plants formed a new root system free of nematodes.

Man uses, in many forms, the principle of mechanical excision of diseased parts as a means of therapy. Removal of localized knots on plums and cherries, and galls on olives and roses are among the more common examples. The systemic wilts are sometimes checked by pruning infected roots or infected branches. Cutting off branches of pear trees several inches below the zone of visible fire blight symptoms is practiced to halt further invasion by the causal bacteria. We have found that removal of the fruits and succulent cane tips of rose bushes stops the advance of *Botrytis* downward through the pith into the crown. The

bark over diseased areas in tree trunks and branches is removed to permit a drier environment unfavorable to the pathogen, e.g., slime flux. For a similar purpose, bark is excised from diseased areas in the exposed main roots and crown of walnuts and chestnuts. Sometimes it is done to permit better contact of toxicant with the pathogen. Bark tracing or removal has been used to check bleeding canker of hardwoods and stripe canker of *Cinchona*. However, the causal *Phytophthora* spp. remain in the roots and may grow upward to kill new sections of the cambium and adjacent living cells.

### B. Temperature Modification

A well-known measure for freeing plants of pests is based upon the differential heat inactivation of the pathogen in, or on, the host. Generally the extent of the injury depends upon the physical state of the respective protoplasm, the degree of hydration or solation. The composition of the protoplasm and the containing membranes also affects the action. When possible, the life activities of the microorganism to be eliminated are stimulated and the host is maintained dehydrated and/or dormant.

Occasionally, the curing of diseased plants by growing at above-normal temperatures is reported. Brierley and Smith (1957) observed that White Wonder and Dynamo chrysanthemum plants infected with the flower distortion virus, when grown in a greenhouse maintained at 35° C. for 2 to 3 months, produced tip scions free of the virus. Heat therapy of viruses is considered in detail by Matthews in Chapter 12 of Volume II.

Hot water immersion, with or without antiseptics in solution, has been used to free crucifer seed of viable black rot bacteria, strawberry plants of virus, and bulbs and rhizomes of nematodes. The differential in heat resistance of host and pathogen may be explained on the basis of the heat stability of their respective enzymes. The thermal growth range of organisms reflects the thermal stability of their cellular proteins. These in turn are affected by the cell water content and the physical and chemical state of the cell components.

### C. Radiation

Ultraviolet radiation is often proposed as a means of plant disease therapy. The researches of Fulton and Coblenz (1929) sum up its advantages and limitations. The shorter wavelengths (about 240 m $\mu$ ) exert the greatest germicidal action, which decreases with wavelength until about 365 m $\mu$  which is an upper limit. The abiotic effect varies with the time of exposure and the intensity of the light. The principal limita-

tion seems to be the inability of the ultraviolet rays to penetrate sufficiently beneath the surface to destroy the thalli of pathogens.

Infrared energy has been exploited for killing microbes in foodstuffs and matériel, but seemingly is not sufficiently selective to be used in plant therapy. Instances where exposure of plants to the visible light spectrum (313  $m\mu$  and longer) has suppressed disease, leave in question whether a direct lethal effect, or the indirect change, is responsible. Ultrasonic inactivation of microorganisms in and on inanimate substrates has been shown, but reduction to practice on living plants is, as yet, not practical.

The therapeutic value of radioactivity as well as its harmful effects on animals are well known. Solutions and powders containing alpha particles were distributed for test on plant diseases by one manufacturer, but no beneficial therapy was evident against Dutch elm disease in our tests. Beta and gamma radiation exert potent stimulatory and lethal action on cell protoplasts but, as yet, nonselectivity, cost, and danger to the user militate against their use in plant therapy.

Scientists of the U. S. Department of Agriculture, according to a recent news release, had hoped that nursery stock and other agricultural products could be freed of plant-pathogenic nematodes by exposing them to ionizing radiation. The golden nematode of potato (*Heterodera rostochiensis*) can withstand radiation up to 20,000 roentgens before the females are sterilized and 120,000 roentgens or more are required for complete killing. Man is a "softie" by comparison, since a mere 300 to 650 roentgens are fatal. Other nematodes require between 350,000 and 640,000 roentgens for a lethal dose. So there is little hope that radiation can be used for killing nematodes in living plants, because nematode-killing doses of radiation also injure plants.

The possibility of using ionizing radiation as a therapeutic agent for a bacterial disease has been explored. Crown gall, caused by *Agrobacterium tumefaciens*, is suppressed by irradiating the inoculated plant with X-rays (Waggoner and Dimond, 1952). Irradiation usually does not kill the pathogen but rather destroys the auxin system of the plant, so that it is unable to respond by gall formation. Thus maleic hydrazide, which also prevents growth of the plant, inhibits gall formation in inoculated tomato plants (Waggoner and Dimond, 1955). Generally, the susceptibility of a fungus pathogen to ionizing radiation is much lower than that of the host. Waggoner and Dimond (1952, 1956, 1957) have studied these interrelations together with the changes in resistance to disease undergone by tomato plants on exposure to X and gamma radiation when plants are then inoculated with *Fusarium oxysporum* f. *lycopersici*. The plants were killed with lower doses than the fungus.



Generally speaking, the effect of radiation treatment on susceptibility of the plant is related to the effect of irradiation on the auxin system.

Potato tubers, irradiated to delay sprouting are more subject than control tubers to rotting by *Erwinia atroseptica* and *E. carotovora* because the treatment delays or prevents periderm formation (Waggoner, 1955).

#### D. Moisture Modification

Intercellular humidity is a factor concerned with disease in a large number of plants. This is most likely where the pathogens are primarily intercellular invaders. Hence, the extent of spread of such diseases within a plant, may be limited, when controllable, by the amount of moisture made available in the environment. This effect of intercellular humidity was demonstrated by Shaw (1935) to exert a definite influence on the degree of fire blight susceptibility in plants of pear and apple. Data indicate, that when 99.5–100% intercellular humidity occurs, maximum host damage results. Conversely, when a turgor deficit, or intercellular humidities of 96% or less obtain, no disease develops. This idea has been validated by Clayton (1937) with another bacterial disease, blackfire of tobacco.

The modification of the moisture-oxygen content of woody stems acts in the therapy of *Verticillium*-infected maple trees by mitigating the symptoms (Caroselli, 1957). Caroselli's data indicate that the degree of injury caused by the fungus is dependent upon the relative amount of moisture and air within the tissues of the tree. When the tree is in full foliage, the water content is least and the air content greatest. Presumably, the additional oxygen favors growth of the systemic fungus so that the wilt symptoms are exhibited after the next rain.

A modern, large-scale commercial application of this measure is the "vacuum cooling" of lettuce and other food crops. The plants are exposed to a partial vacuum. This converts the interior liquid water films to intercellular water vapor which is then withdrawn from the plant structure. This vaporization cools and dries the intercellular spaces. Although thought of as prophylaxis by cooling, a sufficient intercellular drying results to retard the development of bacterial soft rot and other diseases. Thus, Shaw's (1935) hypothesis is reduced to commercial practice.

Intracellular moisture may be manipulated in plants to minimize the extent of disease. Powdery mildew diseases are prevalent in sites where water deficits due to transpiration exceeding absorption often occur daily or for short periods. A possible explanation is that the haustoria of the extramatrical mycelium are unable to invaginate protoplasts exerting a positive pressure. Thus, by consistently making adequate water available

and reducing water loss by one of several means, the spread of mildew can be checked.

A further example of moisture modification and attendant disease damage is the case of golf-green turf and *Curvularia* blight. This may be as much prevention as therapy. On hot, mid-summer afternoons, the close-cut grass ( $\frac{1}{4}$ -inch) wilts due to a water deficit in the leaf blades. This predisposes them to attack by the fungus, which is a common black mold growing on turf debris. Maintaining the turgidity and vigor of the leaf cells by applying a light watering in early afternoon appears to check the advance of the facultative parasite into the healthy tissues.

Endoxerosis or internal decline of lemon fruit was found by Bartholomew (1928) to be caused by exposure of the trees to high temperatures with consequent rapid leaf transpiration when relative humidity of the surrounding air is low. Water is lost from the leaves and in turn withdrawn from the fruit to the point where cellular damage and gum formation occurs. Endoxerosis is most prevalent when growth activities are greatest. Hence, therapy is aimed at maintaining an intermediate rate of growth by manipulation of contributing environmental factors. Blossom-end rot of tomatoes is a similar disease. Likewise, any practice which tends to conserve moisture and provide a uniform supply to the foliage and fruit reduces losses.

Aging celery seed for 3 years often results in "die-out" of *Septoria*. This may well be due to the drying of the seed.

### III. TOPICAL CHEMOTHERAPY

Chemotherapy is one of the exciting new frontiers of plant pathology. Chemotherapy may be topical or systemic.

Ever since Prévost (1807) treated wheat seed with copper sulfate, research on chemical protection of plants against invasion has been going on. Success has been nothing short of fantastic. We have had Bordeaux mixture, wettable sulfur, "fixed" copper fungicides, chloranil, ferbam, nabam, zineb, dichlone, glyodin, captan. The list lengthens daily. Theses have been written, reputations made. Similarly, we have pursued the microbes in the soil with formaldehyde, carbon disulfide, chloropicrin, ethylene dibromide, and 1,2-dibromo-3-chloropropane. This list is shorter, but it lengthens, too. Thus, we have protected our crops. Thus, we have killed the microbial pathogens during the inoculation stage. The contribution of all of these to the science and the art of plant pathology will be treated in Volumes II and III.

During all this time, we have neglected internal therapy. Chemotherapy now gives us hope in the attack on diseases where chemical protection has failed or is impractical. Most of the modern research is

aimed at vascular fungus diseases, at virus diseases, or at bacterial diseases. Few of these have receded before the onslaught of protectants. Perhaps, they will yield to chemotherapy!

Several general and specific discussions on the chemotherapy of plant disease have appeared during the past decade. The potentialities have been covered by Stoddard and Dimond (1949), Crowdy (1952), and Brian (1952a, b).

A compound that initiates a curative or mitigating effect, either directly or indirectly, is termed a "chemotherapeutant." Perhaps an ion or an atom may as well be the active agent as a compound. The pathogen may injure only surface cells or a localized area of tissue. On the other hand, it may systemically injure the entire plant or any major part thereof. Treatment of the former is topical chemotherapy. Treatment of the latter is systemic chemotherapy.

Therapy will advance as we learn how "toxins" act, whether by destroying tissues or by inhibiting or stimulating essential metabolic functions. A key point is the initial reaction that takes place between the invading organism and the host tissue. Such knowledge would permit defining more accurately the specific cellular functions disturbed during the infection period, and would perhaps suggest a therapeutic measure; how to selectively neutralize the pathogen or its metabolic products within the tissues of the host.

Chemotherapy has contributed its part to the subtle realization that only minute concentrations of some chemicals are required to kill fungi and stop disease in plants. Painstaking laboratory techniques and the use of the log-probability curve as a device for estimating the microbial and plant toxicity of compounds have also contributed their part in changing our ideas of biological activity from pounds per hundred gallons of water to parts per million.

#### A. *Therapeutic Index*

Ehrlich of salvarsan fame was surely the founder of chemotherapy as a direct killing action. He emphasized very strongly, indeed, the principle of the chemotherapeutic index; namely, that the dose of the drug needed to kill the pathogen must be below that to kill the patient. The wider the ratio, the bigger the index, and the safer the treatment. In the plant pathologists' jargon, the chemical must not be seriously phytotoxic. This principle dictates the screening techniques to be used and phytotoxicity tests must come early in the procedure.

The literature on the inner therapy of plants has been summarized by Müller (1926). In addition to his own experimentation, there is assembled an orderly, comprehensive account of other investigations on

internal plant therapy before 1926. He repeats the idea that "therapy" is direct control, particularly useful for living endopathogenic and inanimate causal agents. He evaluates chemotherapeutants according to a "Therapeutic Index," which represents the curative dose divided by the tolerated dose ( $I = c/t$ ). Factors affecting the curative dose are pointed out as: (a) kind and condition of the therapeutant, (b) duration of absorption period, (c) desired degree of saturation, and (d) local conditions, i.e., time of year, temperature, air movement, relative humidity, and cloudiness. Factors affecting the tolerated dose by the pathogen (i.e., kind, state of development) and by the plant (i.e., family, morphology, volume, stage of development) are thought of in relation to the strength of the outbreak, the kind of infestation, and the site of the disease.

The topical application of medicaments to surface lesions in order to reduce infection has been practiced for centuries; even though they have been only dung, urea, lime, sulfur, or charcoal. The caustic action of lime sulfur was found to "burn out" the scab fungus (*Venturia inaequalis*) on apple leaves, but often it injured the plant tissue also. Perhaps the largest commercial success of such therapy is the cure of apple scab with water-soluble organic mercurials, introduced as phenyl mercury triethanol ammonium lactate ("Puratize") by Howard and Sorrell (1943).

Another commercial success is the curing of cereal seed invaded by the smut fungus (Tisdale and Cannon, 1929). Success has been reported for the cure of cedar rust by Strong and Cation (1940) with sodium dinitrocresylate. Ark (1941) successfully treated bacterial crown gall with the same chemical. Therapy by the surface treatment of tree branches with chemicals that permeate the bark has been practiced for many years. A zinc chloride-glycerine-alcohol preparation has been used in California for the control of bacterial blight of pear. Coal tar oils are applied in Poland to check brown rot (*Sclerotinia* spp.) cankers on stone fruit trees.

Vegetative parts of plants used for propagation are commonly dipped or soaked in solutions of antimicrobial agents to rid them of established pathogens. An example once widely used is the treatment of seed potato tubers by immersion in solutions of formalin or mercuric chloride. While fairly effective as a curative measure, the phytotoxicity is high and the general presence of *Rhizoctonia* sp. and/or *Streptomyces scabies* in fertile soils militates against its use.

The seeds of many plants (Chen, 1920) are infected internally with bacteria or fungi and the practice of destroying the pathogen *in situ* has generally been referred to as disinfection or disinfestation. Mer-



curials, particularly organic compounds, are most widely used because of their vapor phase activity. Dithiocarbamates, aryl quinones, phenols, and formaldehyde are also used as slurries, dips, or dusts.

Plain water alone is credited with curing disease. Tyner (1957) reported that loose smut, caused by *Ustilago nuda*, can be eliminated from barley by water soaking. He suggests that the effect is not due to microorganisms or their chemical products accumulating in the soak water, but rather to quinones formed in the germ of wheat or barley during the soaking.

Accelerating the healing of wounds by stimulating callus formation through surface application of chemicals has been attempted for many years. Lanolin was found to improve the initial stimulation of healing by Shear (1936) and was further enhanced by addition of such growth-regulating compounds as 4-chloro-3,5-dimethylphenoxyacetic acid and 2,4-dichlorophenoxyacetic acid (Crowdy, 1953). Crowdy found little evidence that the duration of the healing period can be extended markedly by chemical treatment or that out-of-season healing can be stimulated in this way. The improved healing may be due either to an earlier start of the healing process, or to an accelerated rate. Timing of chemical treatment with normal growth activity of the plant seems very important.

#### IV. SYSTEMIC CHEMOTHERAPY

##### A. General Concept

Internal systemic medication is the real frontier of therapy. This challenges us so much because it offers hope of chasing a pathogen into the farthest leaflet of the plant and killing it. The entomologists are ahead of us here. They can kill a leaf miner in the highest leaf of a birch tree by spreading an ounce or so of an organic thiophosphate over the soil in the spring. We must some day be able to repeat this astonishing performance for the Dutch elm disease. Perhaps we can console ourselves in our slowness by saying that the thiophosphate acts as a nerve poison on the insect larva and that trees have no nerves. Our fungus, *Graphium ulmi*, has no nerves either and, hence, is not damaged.

We must not forget the ancient and hoary principle in human medicine that every disease has its specific remedy. We researchers in chemotherapy are aiming at that objective. This subject has been neglected hitherto; because individual plants have been considered expendable, because the problem is intensely complex, and because we did not have access to the formidable array of chemicals and antibiotics now available to us.

In this chapter we shall not be describing, however, very many

practically useful chemotherapeutants. We shall concern ourselves with the nature of the problem, with some of its pitfalls that we have noted, and with some of the challenges. Other facets of the subject have been considered recently by Dimond (1959) and by Dimond and Horsfall (1959). We hope to suggest that "the water is fine" and to encourage others to try a swim. We hope to shed some light on the problem posed by the old hymn: "Watchman, tell us of the night, what its signs of promise are."

### *B. Nutritional Imbalance*

Between therapy by physical measures and what is generally thought of as chemotherapy, is the well-established principle of correcting nutritional disorders of the plant cell. Most commonly, plant disease is thought of as being caused by animate pathogens. However, therapy can and has been, consciously or unconsciously, applied to correction of inanimate causes of disease—those nonliving factors that affect adversely the natural or normal functioning of plant cells, namely, imbalance of water, oxygen, and nutrient elements.

Balancing certain elements in the soil environment or in the plant cells can be used as a therapeutic measure. Tomato fruits low in calcium but high in total nitrogen, iron, and copper are more liable to blossom-end rot according to Taylor and Smith (1957). Conversely, supplying the plants with more calcium and decreasing nitrogen levels has reduced the disease. Similarly, the use of sulfate fertilizers rather than chlorides can reduce succulence of tomatoes and hence mitigate damage. This is therapy of nutritional imbalance.

The major nutrient elements necessary for metabolic equilibrium, viz., nitrogen, phosphorus, potassium, calcium, and magnesium, are taken care of by adjustment of fertilizer practices. The minor elements that may induce the so-called "deficiency diseases"—zinc, boron, copper, manganese, sulfur, and iron—are identifiable from a diagnostic key to symptoms prepared by McMurtrey (1948). One has the option of considering as chemotherapeutants the metabolites or nutrients named above, when used to maintain or to improve the normal cellular activities.

After observing the therapeutic effect of zinc on mottle-leaf of *Citrus*, Reed and Dufrenoy (1935) explained the action on the basis that the salts of certain metals catalyze the partial oxidation of sulfhydryl compounds. Certain of these compounds are present in all living cells and control the life processes through maintaining the energy at a given level by oxidation. This level is defined as the oxidation-reduction potential; which when low, may result in pathological symptoms cor-

related with an accumulation of suboxidized metabolic substances as in mottle-leaf of *Citrus*.

Profound changes in the cytological conditions are associated with the recovery of mottled trees after the entry of zinc, applied either to the soil or to the foliage in the form of a spray. Zinc accumulates in the meristematic cells of buds and in the palisade cells of green leaves. After zinc sulfate has been applied, the symptomatic trees resume normal leaf cellular activity as judged by normal nuclei, fibrillar cytoplasm, and normal chloroplasts, and as exhibited by accelerated growth of new shoots. These effects suggest that some reaction has been initiated by which the proteins and carbohydrates of the cells have been utilized to supply energy to the cells. Steroids accumulate in cells of mottled citrus leaves, but are scarce in leaves to which zinc is applied. Seemingly, the stabilizing of the sulfhydryl compounds by zinc promotes the oxidation of cell metabolites and thereby liberates energy for vital processes.

Undoubtedly, zinc is linked with sulfur compounds in maintaining normal green plant cellular functions. Yet, in solution, zinc at concentrations of 5 to 25 p.p.m. has been reported to stop the growth and kill corn and citrus seedlings. How the zinc requirements of plants can be met without toxicity has been explained by Masé (1914). Presumably, in the presence of calcium carbonate, zinc is precipitated as an insoluble salt. Necessary quantities are dissolved out by root excretions and absorbed as required by the plant without toxic accumulation. Thus, zinc carbonate or a similar basic salt would serve as a "safe" or sublethal reservoir of the essential toxic metabolite. This same principle can and has been used with other metal ions in other "deficiency" diseases of plants.

Molybdenum in very small amounts has been reported as needed for the physiological reduction of nitrates in plants (Turner and McCall, 1957). Nitrates accumulate in the tissues of molybdenum-deficient plants, and the protein content of the plant is reduced. This upset metabolism results chiefly in characteristic hypoplastic symptoms, as for example "whiptail" of crucifers. Treatment with a few parts per million of available molybdenum suffices to cure this growth condition.

In contrast, an excess of a single nutrient or a combination of nutrients may directly or indirectly result in injury. Here we get into the area of chemical antagonism and physical changes in the state of the plant protoplasm. Of major importance is the accumulation of sodium salts and resultant "alkali" or "salt" injury. Nevertheless, the removal of an excess injurious salt or element, even modification of soil reaction to within the range for normal plant growth, is a chemotherapeutic practice in the broadest sense.

Healthy living cells are believed to have an approximate balance of hydrogen and hydroxyl ions. They are able to maintain an internal neutrality because of their slight permeability to these ions. The internal pH can be upset by the greater penetration of undissociated molecules of acidic and basic substances than of their corresponding ions. Hence, when the plant cells are exposed to low pH values, weak nonionized acids enter the cells and damage them. Similarly, weak poorly dissociated bases are toxic at pH values above 7.0 because they can permeate the cell at that pH. Hence, correcting the chemical environment will cure the harmful effects.

Feldman *et al.* (1950) observed that the Dutch elm disease organism (*Graphium ulmi*) forms its most toxic metabolite when the pH is 4.2 and successively less as the pH of the medium is raised to 7.0 or above. Therefore, an alkaline formulation containing lime was developed for the impregnation of soil around elm trees, to suppress or avoid the development of symptoms. A check *in vivo* of the pH of the tree sap indicated a rapid rise in alkalinity for a few days following soil application and then a dropping back to normal. The experimental results suggest that some curative effect was attained; either from the pH modification, or the high calcium hydrated lime and other ingredients used.

### C. Biochemical Specificity

The idea of finding a chemical that may be used as a systemic medicine is based on the principle of biochemical specificity as instanced above for the birch leaf miner. The idea of biochemical specificity, perhaps, goes back a century to the recognition by Louis Pasteur that microorganisms are so specific in their biochemical reactions that they can separate optical isomers. Ehrlich (1913), 50 years later, seems to have had in mind selectively active substances when he established the term "chemotherapeutics." Dubos (1958) has stated recently that "under natural conditions, each one of the microbial species concerned in the economy of organic matter, is more or less specifically adapted to the performance of a limited, defined biochemical task."

The concept of chemotherapeutic specificity has been sharpened over the years. Formerly it was considered on the basis of the whole cell, later on the basis of the enzymes, and more recently on the basis of the molecular structure of the substrate.

Dubos (1945) has pointed out that effective therapeutants do not behave as gross protoplasmic poisons which affect indiscriminately the structure and function of all living cells. They interfere selectively with some specific steps concerned in the nutrition, synthesis, or cell division of the pathogen. Other therapeutic agents may react selectively with



well-defined structural components essential to the pathogenic behavior of the cell. This attitude is hopeful for the inhibition of pathogens within the tissues of the host plant.

Yarwood (1955) has presented evidence of "selective accumulation." Although the lethal dose of fungicide per unit of tissue be the same for host and fungus, the fungus may accumulate the toxic agent in larger amounts than the host, and is, therefore, killed at a lower applied dosage than the host. Autoradiographs indicate that this is true for sulfur therapy of rusts. Also there may be "selective toxicity." The amount of fungicide accumulated per unit of tissue may be the same for host and fungus, but the fungus is killed at much lower accumulated dosages than the host. This seems to apply to the sulfur therapy of powdery mildews.

The possibility of finding chemotherapeutants exerting a specific action is strengthened by what has been learned from penicillin. Penicillin presumably blocks the synthesis of a particular wall component of the bacterial cell, but not that of the host. Thus, its action is specific. If growing bacterial thalli are unable to form cell membranes, they undergo osmotic lysis. Undoubtedly, compounds with similar action are waiting to be found for plant chemotherapeutants.

Enzymes capable of specific lysis of fungous structures are sometimes to be found among fungi. As yet, knowledge of these enzymes has not been developed for use in plant therapy. The plasmodia of some Myxomycetes have been observed to destroy the hyphae of fungi with the same apparent speed that a hot iron may cause nylon or acetate fibers to melt. The inky cap mushrooms demonstrate the property for all to see.

Perhaps a better suggestion of possibilities for future specific agents is offered by the diplont or dicaryon stage of certain Ascomycetes. In the so-called sexual phase, a changed metabolic pattern follows differentiation of "fertile hyphae," an ascogonium, and ascogenous hyphae from the somatic tissues of the thallus. This diplont exerts a cannibalistic action on the haplont matrix of the surrounding stroma. This action has been used as an ordinal taxonomic character. It seems possible that the lytic enzymes, produced by the diplont to make room for itself in the body of the haplont, could be isolated and provide the key information required to discover a specific chemotherapeutant useful in plant therapy.

#### *D. Microbial Disease Therapeutants*

##### *1. Intake or Entry*

Clearly we must start with the problem of intake or entry of chemotherapeutants. Our medical colleagues call this "route of admin-

istration." Through what routes can systemic therapeutants enter the plant? There are really only three practicable routes of administration for plants—root, stem, foliage. Mechanical injection is possible through each. Roach (1939) has published a basic treatise on the injection of materials and their subsequent distribution in the plant. Each portal of entry has its advantages, each its drawbacks.

Roots grow in soil. A compound aimed at roots must go first through the soil and be subjected to its severe subtractive influences: distance, adsorption, direct chemical alteration, and biological degradation.

The significance of distance is obvious. The farther the compound must go before it can reach the roots, the less will reach the roots. This follows from the simple laws of diffusion and dilution and is not of necessity related to any loss along the way due to hazards of the route.

Adsorption is a much more serious source of trouble. If a substance bears a strong charge, it will be adsorbed onto soil particles, presumably by van der Waals forces, and it may never arrive alongside the root to take it. Streptomycin bears such a charge according to Siminoff and Gottlieb (1951) and, thus, it is not an effective chemotherapeutant when applied to the soil. The same is true of thiolutin according to Gopalkrishnan and Jump (1952) and of numerous other antibiotics according to Martin and Gottlieb (1955). Ark and Alcorn (1956) showed some specificity in adsorption. Streptomycin is adsorbed more tightly by bentonite than by pyrophyllite. When Ark and Alcorn changed the charge on the clays with  $K_2HPO_4$  the streptomycin was released.

Since soil is a reasonably reactive medium, it decomposes many candidate chemotherapeutants and reduces them to impotence. Our knowledge of this is pretty limited, but what we have is clear. Antibiotics seem to have provided more information to date than other types of therapeutants. According to Jeffreys (1952), many antibiotics such as albidin, gliotoxin, and viridin come apart in soils where the pH is unfavorable. Even if the pH is not unfavorable, some antibiotics such as penicillin and streptomycin break down.

The soil is so full of microbes that it is not surprising that they, too, degrade chemotherapeutants very rapidly. Very few molecules have demonstrated radical resistance to microbial decomposition. DDT is an example, but, of course, it is not antimicrobial. Very probably the two are related. If DDT reacts with microbes to damage them, then the microbes will react with DDT and damage it. Thus, we fight an uphill battle when we introduce antimicrobial substances into a milieu loaded with microbes.

Jeffreys (1952) has shown that antibiotics such as griseofulvin, patulin, and mycolic acid will survive longer in organism-free soil than in a natural soil full of microbes.

Walker and Smith (1952) show that *Myrothecium verrucaria*, a very widespread cellulose decomposer, can actively destroy cycloheximide (Actidione). Salicylic acid analogues have been shown to contain some systemic chemotherapeutic properties on bacterial blight of bean (Diamond *et al.*, 1952), but Riere (1940) has shown that *Aspergillus*, a common soil organism, can utilize salicylic acid as a source of carbon.

Wright and Grove (1957) have recovered a *Pseudomonas* species from soil that can degrade griseofulvin. This bacterium increases steadily in the soil as griseofulvin is successively added to the soil.

Gottlieb (1957) has published an excellent review of the microbial decomposition of toxicants. We can only conclude that chemotherapeutants destined for entry into the plant through the roots must run a very hazardous gauntlet. This inevitably suggests that screening methods for chemotherapeutants should include a "soil burial" test analogous to that used by our colleagues in wood and fabric degradation.

Even assuming that the candidate therapeutant survives the hazards of the soil and arrives alongside the root, it must pass through the epidermis of the root, migrate through the cortex, if necessary through the endodermis, and finally, into the free-flowing stream in the xylem. This formidable collection of barriers further separates the "sheep from the goats, the men from the mice." The number that survive falls fast. Here also adsorption plays its accustomed role.

Of course, investigations on root entry are best done when the roots are placed in sand or in solutions or suspensions of the test substance, not in soil.

Chapman (1951) has reported on the comparative performance of 8-quinolinol and *n*-octadecyltrimethylammonium pentachlorophenate. The former is almost without charge. It does pass the root barrier and go up the stem of an elm (Zentmyer *et al.*, 1946). The latter compound is highly charged, is substantive to cellulose according to Chapman (1951), and it does not move out of the roots.

The relative performance of these two therapeutants is striking. The former gets into and up the stem of elms to the point where invasion by the Dutch elm disease fungus occurs high in the tree. The latter compound is utterly ineffective on Dutch elm disease, but is reasonably active on *Fusarium* wilt of tomato where invasion occurs through the roots. This compound stops in the roots and is effective there. The other apparently does not stop there and is ineffective there.

In 1947, Anderson and Nienow demonstrated that streptomycin enters roots of soybeans. It also enters the roots of peach (Dye, 1956), cucumber (Pramer, 1953), broad bean (*Vicia faba*) and tomato (Pramer, 1954).

Chloramphenicol can also enter roots, whereas chlorotetracycline,

oxytetracycline, and neomycin can not (Pramer, 1954). The evidence is clear that the following other compounds can enter the plant through the roots: cycloheximide (Wallen and Millar, 1957), griseofulvin (Crowdy, 1957), thiolutin (Gopalkrishnan and Jump, 1952), sulfonamide analogues (Dimond *et al.*, 1952; Crowdy and Rudd Jones, 1956), 4-chloro-3,5-dimethylphenoxyethanol (Davis and Dimond, 1953), benzo-thiazolyl-2-thioglycolate (Dimond *et al.*, 1952) and many others.

The charge on streptomycin affects its adsorption into root tissue just as it affects adsorption onto the soil particles (Pramer, 1954). Streptomycin tends to remain in broad bean roots just as *n*-octadecyltrimethylammonium pentachlorophenate does, but to a lesser degree. Pramer's work also suggests that chloroamphenicol and griseofulvin are not retained in the roots; presumably, they are not heavily adsorbed there.

Cycloheximide does not seem to be heavily adsorbed onto root tissues (Wallen and Millar, 1957).

Crowdy and Rudd Jones (1956), like Chapman (1951), have related the adsorption of sulfonamides by root tissues to their adsorption on cellulose. Their techniques were somewhat more sophisticated, however. They have shown that the rate of entry of sulfonamides into broad bean roots is related to their  $R_F$  values (the ratio of the distance traveled by the material to the distance traveled by the solvent) on cellulose chromatograms. This looks like a smart approach to the problem.

The ratio of entry to the  $R_F$  value on cellulose is a constant for the neutral compounds that are fat soluble, but the more water soluble the compound, the higher the proportion of aqueous phase in the chromatograph system needed to maintain the same ratio. Apparently, neutral compounds and those with high pKa values (pH where ionization is least) enter the plant as undissociated molecules, but those with low pKa values enter as ions. Of course, when the pKa value is near 7, pH is very important in dissociation and, hence, is closely associated with entry of the compound.

Bearing in mind that systemic chemotherapeutants must move through living cells of the root before entering the xylem, one is not surprised to note from the literature that the process requires energy as many permeative processes do (Brian *et al.*, 1951, and Stokes, 1954). Griseofulvin shows an initial rapid entry into wheat roots and this is inhibited by such respiration inhibitors as nitrophenol, phenylurethane, and sodium azide. At the concentrations used, these inhibitors do not inhibit the entry of water, however, according to Crowdy *et al.* (1956). If the inhibitors were applied for a prolonged period, some griseofulvin did enter the root presumably with the uninhibited water of the transpiration stream.

Crowdy and Rudd Jones (1956) pursued this line of research also



with the entry of sulfonamides into the broad bean plant. Some of the sulfonamides enter readily, some do not. Those that do, act similarly to griseofulvin. The initial spurt in the entry is sensitive to respiration poisons, but the long time slow entry is not. The latter is related to the rate of entry of water.

Presumably, one could force candidate therapeutants through the roots, but we have no information on such trials.

Much research has been done on entry through wounds in the stem. Stoddard (1947) forced his test compounds into the severed upper ends of the stems of peach seedlings. He seems to have had no followers, but his methods would seem to warrant further exploration.

The uptake of chemicals by stems through bore holes has been very popular. This technique has been resorted to largely because it permits estimation of the dosage within the plant. Howard (1941) used the method for his work on diaminoazobenzene for bleeding canker of maples, and so did Zentmyer *et al.* (1946) in their earlier researches on Dutch elm disease.

Radial bore holes were used at first and the compounds used were water soluble. Later, Howard and co-workers (Anonymous, 1955) developed a method of injection that depends upon tangential bore holes arranged in an ascending spiral around the tree. The holes are filled with the compound in paste or powder form and sealed. The tangential boring assures that the chemical is accessible to the maximum number of xylem vessels and the "dry" packing assures a longer lasting supply of chemical. The method, when properly timed, seems to be successful with 4,5-dimethylthiazolyl-2-thioglycolate for Dutch elm disease, but not for 8-quinolinol benzoate.

Is a tree "cured" of disease when the development of symptoms is stopped? For example, a 25-year-old elm tree about 8 to 10 inches in diameter at breast height became naturally infected with *Graphium ulmi* as proved by tissue culture from wilted terminal branches. Immediate chemical treatment upon sighting the symptoms checked further wilt. Subsequently, the dead branches were removed and the tree appeared healthy (symptomless) for 2 years until another attack developed. This was confirmed as due to *Graphium ulmi* by laboratory diagnosis. Again prompt treatment by the bore-hole method resulted in checking the disease and it has remained "healthy" for 3 years.

In another case, an elm was proved to be infected with *Graphium ulmi* by isolation of the fungus from branches bearing wilted leaves. It was promptly treated by soil impregnation of the rhizosphere and a further development of symptoms was stopped. The next two seasons, normal foliage appeared on undamaged branches and the killed ones

were cut off. However, discolored streaks in the xylem, formed during the year of active disease, were separated from the cambium by annual layers of new wood. This discolored wood yielded pure cultures of the pathogen. Thus, it would appear that a sometime pathogenic fungus can continue to exist in a quiescent state in a host plant without causing injury. There may be a "walling-off" effect brought about by the interposition of cells having thicker walls or unfavorable chemical composition, or the metabolic environment is not favorable for pathogenesis.

To test this, ax cuts were made through the bark and into the discolored area harboring the "quiescent" pathogen, with the result that in some cases symptoms again developed. Beckman (1958) now believes that arresting Dutch elm disease by 4,5-dimethylthiazolyl-2-thioglycolate treatment creates an unfavorable physiological environment, rather than a mechanical barrier for walling-off the fungus.

Sometimes severed stems are used in exploratory work on chemotherapeutants especially in studies on host tolerance. Robison *et al.* (1954) have shown that antibiotics enter more readily through cut stems than through roots, but this, of course, is not unexpected.

Some work has been done on entry through intact stems. This is a technique first used by growth hormone investigators. Mitchell *et al.* (1952) applied streptomycin to bean stems by mixing it with lanolin and a nonionic detergent. Streptomycin will also enter the intact stems of tobacco (Hidaka and Murano, 1956) Polychlorobenzoic acid (Beckman, 1959) will readily enter when painted on the bark of young elms and affect the development of the Dutch elm disease.

If we could find or tailor-make chemotherapeutants that enter readily through intact foliage, we would be a long way toward a practical solution to the entry problem. If the compound enters the foliage, it is very near to the site of action of all above-ground diseases. It need not be translocated very far, and it can be easily and cheaply applied as a spray or, perhaps, as a dust.

Many compounds do enter through intact foliage; some directly through the cuticle and others through natural openings. This problem also has been pioneered by the growth-hormone investigators. The problems are similar to those encountered in root entry. The compound must pass through the epidermis, through the parenchyma tissue, and into the xylem or the phloem. The problem is further complicated by the presence of the waxy cuticle which is pretty inert. It is eased by the absence of soil and its complications and it is eased by the presence of stomata and hydathodes. Curtis (1943) has shown that compounds can enter through the hydathodes where guttation drops form.

Crafts (1948) has contrasted the types of compounds that might

enter through foliage or through roots. He feels that compounds that enter the leaf should be somewhat less polar and more lipid-soluble than those that enter roots.

A mixture of aluminum sulfate, kaolin, and calcium carbonate, (Cuneo Mixture) administered either to the foliage or in proximity to the roots of trees or herbaceous plants, is claimed (Mosca, 1958) to act as a systemic fungicide for several diseases. Mosca believes that all polar (+ charged) compounds of metal derivatives conductive of electricity have fungicidal properties. Their fungitoxicity is proportional to the degree of dissociation. When a polar substance is dissolved in water, it decomposes into atoms furnished with electric charges (free ions)—the ionization of polar substances is spontaneous. According to Mosca (1952),  $Al^{+++}$  in the ion state acts as a "systemic fungicide with a universal action," because the aluminum ions are conveyed by the protein substances throughout the plant.

Of course, it is now common knowledge that phenoxyacetic acid analogues penetrate foliage very easily.

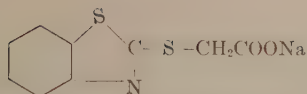
It is interesting to note that sodium benzothiazolyl-2-thioglycolate penetrates elm foliage and has definite therapeutic effects on Dutch elm disease (Dimond *et al.*, 1952). This compound has an  $-S-CH_2COOH$ , which is the sulfur analogue of the  $-O-CH_2COOH$  side group of 2,4-D (Fig. 1). Similarly, van der Kerk's (1956) new systemic fungicide has precisely the same group attached to it. This compound is disodium ethylene bisdithiocarbamyl-dithioglycolate. It is also interesting that captan has a similar tail ( $-S-CCl_3$ ) attached to the imide grouping. The  $-S-CCl_3$  has the important similarity of  $-S-CH_2COOH$  in that the electronegative groups  $C=O$  and  $Cl_3$  are separated by one carbon from the sulfur.

Captan does not enter intact foliage as easily as sodium benzothiazolyl-2-thioglycolate or 2,4-D, but it does seem to possess local chemotherapeutic properties as Stoddard (1954), and Rich (1956) have shown for cucumber scab. It would appear that  $-S-CH_2COOH$  is a better "shaped charge" (Horsfall, 1956) than  $-S-CCl_3$ .

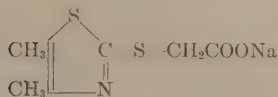
These results suggest that, perhaps, permeation and, perhaps, translocation are encouraged by  $R-S-R'$  or its oxygen analogue.

When one surveys some of the known synthetic chemotherapeutants, several structures of this type appear as shown in Fig. 1. The R seems to vary but apparently the R' should contain an electronegative group.

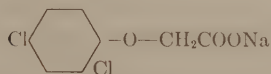
From these structures, one can see others that perhaps should be tried. Nirit, the protective fungicide, is 2,4-dinitrophenylthiocyanate. Here the cyanate group provides the electronegativity. Perhaps, it would be therapeutic. Perhaps, substitution of chlorines for the nitro groups



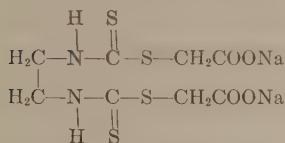
Sodium benzothiazolyl-2-thioglycolate



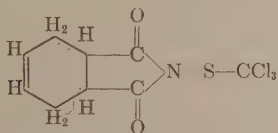
Sodium 4,5-dimethylthiazolyl-2-thioglycolate



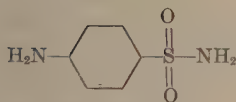
Sodium 2,4-dichlorophenoxyacetate



Disodium ethylene bis-dithiocarbamyl-dithioglycolate



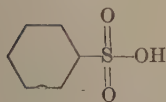
4,5-Cyclohexene-1,2-dicarboximide (captan)



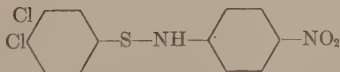
Sulfanilamide



Calcium sulfamate



Benzene sulfonic acid



N-(4-nitrophenyl)-3,4-dichlorobenzene sulfonamide

FIG. 1. Permeating structures.



would make it a close analogue of 2,4-D and, perhaps, improve its activity.

Antibiotics also enter uninjured plant leaves. Davis and Rothrock (1956) demonstrated that griseofulvin applied to one surface of tomato leaves controlled *Alternaria solani* applied to the other. Similarly, Dye (1956) applied streptomycin to the lower surface of peach leaves and protected them against *Pseudomonas syringae* applied to the upper surface.

A few data are available on the weather factors that influence entry through intact foliage. The longer the applied material remains damp the longer the time available for entry. Thus, high humidity delays the drying and promotes entry as Dye (1956) has shown for streptomycin. This principle has been exploited by Gray (1955) to improve the entry of streptomycin. He used glycerine in the water drops of streptomycin to delay drying. It improved the entry.

Goodman (1954) has used Cellosolve and Carbowax 4000 for the purpose of increasing the entry of streptomycin and oxytetracycline for fire blight control on apple and pear.

Glycerine sometimes does not improve entry as Shaw *et al.* (1957) have shown for tobacco foliage and Rich (1956) for cucumber foliage.

## 2. Translocation

If we are to attain systemic chemotherapy, we must attain excellent translocation from the point of entry to the point of need. If we are concerned with leaf spots, we must obtain long distance translocation upward if we use root application. If we are concerned with root rots, we must obtain long distance translocation downward if we use foliage application. Conversely, foliage application is more likely to succeed for foliage diseases and root application for root diseases. Vascular diseases and systemic viruses are hard to reach from either end.

We in plant pathology are more hampered than our medical colleagues by translocation problems. An antibiotic injected into an artery or into a vein will appear in all parts of an animal in a fraction of a minute. The plant, unfortunately, has no such circulation system. And what circulation system it has occurs in microscopic units. We have no tubes as big as the arm vein, for example. We cannot inject very well the whole body from a single point.

The upward system is different from the downward. By and large upward translocation seems relatively simple. Many compounds applied to the roots will appear pretty well all over the tops, but compounds applied to the tops often do not travel downward at all. One day this problem will have to be solved. Delay in its solution will delay the success of chemotherapy of plant diseases.

The assay of translocation is seldom simple. A few antibiotics produce characteristic biological effects. For example, griseofulvin produces a characteristic curious curling of the germ tubes of *Botrytis allii*. Its presence can, thus, be detected and has been so used by Brian (1952a) and his associates.

The bacteriologists have developed strains of *Escherichia coli* that must have streptomycin or they die. If streptomycin-treated plant tissue supports their growth one can be reasonably sure that streptomycin is there. Mitchell *et al.* (1953) have studied streptomycin uptake and translocation with this dodge.

Van Raalte (1952) has devised a partial answer to translocation. He applies the test compound to a cut end of a short piece of potato stem standing on an agar plate seeded with bacteria or other organisms sensitive to the test compound. A zone of inhibition indicates translocation.

Phelps *et al.* (1957) have shown that cycloheximide, cycloheximide acetate, and oligomycin travel in the xylem if injected into the stems of oak trees. Robison *et al.* (1954) have found that antibiotics vary greatly in xylem movement even if they enter unhindered through cut stems. Presumably, this is the ancient old bugaboo of adsorption to the cellulose. The tetracyclines move upward much more rapidly than streptomycin and neomycin. We have already learned, however, that the streptomycin molecule is charged and that it is readily adsorbed. We have seen that  $K_2HPO_4$  will elute streptomycin from clays. The same authors (Ark and Alcorn, 1956) also have shown that  $K_2HPO_4$  will keep streptomycin moving in the xylem elements of *Pyracantha* cuttings.

Streptomycin appears to move in the xylem more rapidly in the peach (Dye, 1956) than in some plants because the concentration in the leaves rises above that in the roots. In the broad bean (Primer, 1954) streptomycin concentrates more in the roots than in the leaves. Griseofulvin and chloramphenicol move steadily out of the treated broad bean roots and accumulate in the foliage. Griseofulvin acts in tomato, according to data of Crowdy (1957), like streptomycin does in the broad bean. There is a gradation from the roots right out to the highest leaves. It must be bound by the first sites and moves further only as the lower sites become saturated.

There seems no doubt that many of these compounds tend to move in the xylem because some of them show up in the guttation drops that exude from the hydathodes, i.e., cycloheximide (Wallen and Millar, 1957), and griseofulvin (Brian, 1952a). Other evidence is that entry of some antibiotics over the long pull or in the presence of respiration inhibitors is proportional to the amount of water lost in transpiration (Crowdy *et al.*, 1956).

On the other hand, Hidako and Murano (1956) concluded that if

streptomycin is applied to tomato stems it moves in the pith and phloem, not in the xylem.

Napier *et al.* (1956) applied streptomycin to the simple primary leaves of bean plants and observed an effect on halo blight as far away as the fourth trifoliate leaf.

Crowdy and Pramer (1955a, b) summarized data on translocation. They concluded that neutral or acidic compounds such as griseofulvin move readily in the plant, that basic compounds such as streptomycin and amphoteric compounds such as the tetracyclines give different results with different plants.

Our ignorance of the field is profound.

### 3. *Host Action on the Therapeutant*

No host will sit idly by when it is treated with a therapeutant. It may detoxicate a therapeutant, enhance its effect, or possibly even excrete it through the leaves or roots. Hilborn (1953) has presented a tantalizing abstract showing how different hosts affect chemotherapeutants. We regret that he has not elaborated it. He shows, for example, that chloramphenicol reduces verticillial wilt of potato, but not of tomato.

a. *Detoxication in the Host.* We have already seen how soil microbes may destroy a chemotherapeutant. Likewise, the living host also may degrade a chemotherapeutant and render it impotent. This is a very well known phenomenon in human medicine and much is known of the mechanisms of degradation.

In plant chemotherapy, however, about all we know is that some compounds must be degraded because their effect is lost. 8-Quinolinol derivatives are evanescent in the plant. The sulfate lasts a few days, the benzoate a few days longer. We do not know what happens to them, but perhaps they are degraded.

The term "half-life" is common these days to quantify the rate of loss. One may, thus, rate chemotherapeutants in terms of half-life. Some of the systemic phosphate insecticides have a half-life that can be expressed in weeks, but most microbial chemotherapeutants have half-lives measured in days, perhaps even in hours.

Brian (1952a), for example, showed that griseofulvin has a half-life of only a few days. Crowdy (1957) has determined this number to be about 4 days. Prescott *et al.* (1956) have shown that cycloheximide has a half-life of about 24 hours in the cherry fruit but much longer in cherry foliage. According to data of Wallen and Millar (1957), cycloheximide may have a half-life in wheat foliage of 2 or 3 weeks. Data of Hidaka and Murano (1956) suggest that the half-life of streptomycin in tobacco foliage also may be as much as 2 or 3 weeks.

In the human body, acetylation is a very common device for detoxicating drugs. Esterases are common among most living organisms. One would expect that higher plants would be able to make esters out of poisonous substances. Unfortunately, only a few have searched for such reactions. Rudd Jones and Wignall (1955) have shown, however, that the broad bean can acetylate sulfanilamide. Undoubtedly, many more cases of such reactions await discovery. We shall return to this reaction below in discussing possible means of producing effective chemotherapeutants.

Other devices are undoubtedly available to host plants in their fight against a foreign chemical, the introduced therapeutant. Gottlieb (1957) has discussed how microbes can detoxicate toxicants. Some, perhaps all, of these mechanisms are available to the host. He mentions hydrolyses, esterification, chelation, direct reaction with metabolites, oxidation and reduction, and displacement effects. We need much more research in this field. Sanwal (1956) has shown that fusaric acid is decarboxylated by the tomato plant and transformed into a nontoxic substance.

b. *Activation by the Host.* The host can react with a compound to enhance its effect, just as it can react with a compound and reduce its toxic effects.

Martin (1950) showed fairly early that the systemic insecticide bis-(bisdimethylaminophosphonous)-anhydride is more toxic after it has been acted on by the host than before. Similarly, the effectiveness of 4-chloro-3,5-dimethylphenoxyethanol to reduce fusarial wilt of tomato improves with time (Davis and Dimond, 1952).

This type of evidence is not conclusive proof that the compound actually changes. The improved effectiveness with time could be due to production of a more effective compound or to an increase in the resistance of the host. The phenoxyethanol probably is oxidized in the plant to phenoxyacetic acid. Since, however, the effects of phenoxyacetic acid also increase with time, one is more inclined to suggest as did Davis and Dimond (1953) that the resistance of the host is increased as well.

Gray (1958) has evidence that tobacco tissue converts streptomycin amine and streptomycin oxime to a more toxic compound, probably into streptomycin itself or dihydrostreptomycin. Similarly, Lemin and Magee (1957) have found that cycloheximide acetate is deesterified by the host to cycloheximide itself. Rombouts and Sijpesteijn (1958) suggest that the cucumber and broad bean may convert the nonfungitoxic carboxymethyl derivative of pyridine-2-thiol-*N*-oxide back to the fungitoxic thiol compound.

Van der Kerk (1956) has synthesized the carboxymethyl ester of nabam. It has the following structure:  $\text{HOOC}-\text{CH}_2-\text{S}-\text{C}(\text{S})-\text{NH}-$



$C_2H_4-NH-C(S)-S-CH_2COOH$ . In this form it enters the plant readily. Inside the plant, it seems to be reconverted to the fungicidal substance, nabam, by deesterification.

All of these results suggest ways of producing new chemotherapeutants and some of these will be discussed below.

c. *Excretion from the Host*. Our medical colleagues have made great strides in learning how chemotherapeutants are lost from the host by excretion. Indubitably, plants also excrete chemotherapeutants but our knowledge of the subject is meager indeed.

Presumably, the compounds can be excreted through the leaves or through the roots. In the leaf, the most obvious excretory organ is the hydathode. Some chemotherapeutants have been recovered from guttation water. Notable among these are griseofulvin (Stokes, 1954) and cycloheximide (Wallen and Millar, 1957). Gopalkrishnan and Jump (1952) were not able to recover thiolutin in the guttation water.

The idea that roots excrete all manner of compounds has only recently been given detailed consideration. Sadasivan and Subramanian consider all aspects of this fascinating subject in Chapter 8 of Volume II of this treatise.

We must have more knowledge about root excretion of compounds if we are to combat root rot diseases by spraying the foliage. Halleck and Cochrane (1950) and Zentmyer (1954) have made a start to search for compounds that could be applied to the foliage, translocated to the roots, and excreted into the soil. This would appear to be a promising lead to follow.

## V. MODES OF CHEMOTHERAPEUTIC ACTION

Having found an effective therapeutant; having preserved it from degradation by soil, microbes, and host; having found that it is translocated to the infection court; having saved it from excessive excretion, what do we know about its mechanisms of action?

Research so far suggests three major mechanisms of action: vivotoxins are destroyed; the pathogen is killed by direct action; or the resistance of the host is enhanced. These we shall now discuss.

### A. *Inactivating Vivotoxins*

Plant pathogens excrete sewage products into the medium where they grow. Sometimes these are called "staling products," because they inhibit the very fungus that excretes them.

Plant pathologists, seeing such effects in fungus cultures, have suggested for a half century or more that such products must also be produced by the fungus in the invaded plant and must participate in the pathogenesis of disease. In such cases the substances are called toxins.

This has always been an intriguing hypothesis. It is discussed in all of its implications by Ludwig in Chapter 9 of Volume II of this treatise.

Dimond and Waggoner (1953) insisted that the alleged substances must meet Koch's rules of proof, if they are to be implicated as causal factors in disease. For one thing, they must be recovered from the diseased host. It is not enough that they be found in laboratory media.

Those that can be recovered from the hosts were labeled vivotoxins.

Since vivotoxins probably do occur in the chain of causality, one is intrigued by the possibility that pathogenesis can be reduced by neutralizing such toxins. Howard (1941) first proposed such a possibility to account for his alleviation of the symptoms of bleeding canker of maple with diamino-azobenzene. Zentmyer (1942) and Stoddard (1946) offered a similar explanation for the action of 8-quinolinol in alleviating symptoms of Dutch elm disease. In neither case was a vivotoxin proved.

Perhaps the best example of a vivotoxin is that of the wildfire disease of tobacco as investigated by Braun (1950) and by Woolley *et al.* (1952). The vivotoxin is a complicated amino acid analogue of methionine and it can be antidoted chemotherapeutically with methionine.

A very new comer as a vivotoxin is fusaric acid which clearly is involved in the pathogenesis of fusarial wilt (Lakshminarayanan and Subramanian, 1955). It is an analogue of pyridine. Subramanian (1956) showed that its effects could be antidoted with 8-quinolinol thus putting props under Horsfall and Zentmyer (1942) who had said that 8-quinolinol seemed to mitigate the symptoms of Dutch elm disease by antidoting a toxin.

However entrancing the vivotoxins may be in the genesis of disease, their treatment by chemotherapy may be a delusion. As long as the pathogen remains in the tissue it will presumably continue to excrete the vivotoxin. That means that any antidoting chemical must be maintained continuously in the system. This will be difficult. One is in the position of Alice in Wonderland. He must run as hard as he can to stay even.

Horsfall (1956) says that he had had hopes that perhaps the destructive action of the toxin could be kept under control long enough to enable the host to throw off the invader by other means. As yet, this still remains only a hope.

The future looks dark for successful chemotherapy by toxin control alone.

### B. *Direct Action on the Pathogen*

If the microbe is a primary causal factor in disease, surely we must kill it out if we are to save the host. Success in so doing is very elusive, however. For many years after the modern reactivation of research on

chemotherapy, the number of cases of direct killing were scarce indeed.

Before proceeding to a discussion of such successes as we have had, we should consider direct killing of the pathogen by substances found in nature. These, perhaps, will be useful to suggest further research on synthetic substances.

### 1. *Natural Therapeutants*

Plants display a wide variety of cases where resistance to disease seems chemical in nature. This matter is discussed in considerable detail by Allen in Chapter 12 of this volume and need only be referred to here.

The role of phenols and tannins in natural resistance has been discussed for years. The classic example is protocatechuic acid which has been shown by Walker *et al.* (1929) to be responsible for the resistance of red onions to smudge. Natural therapeutic agents, such as phenolic compounds, have been found in trees by Rennerfelt (1945), and Erdtman (1949).

Rich and Horsfall (1954) have called attention to the fact that quinones are generally more fungitoxic than phenols. Schaal and Johnson (1955), investigating the role of chlorogenic acid in resistance of potato to scab, suggest that the phenol is oxidized to a quinone before maximum resistance is attained. Presumably, this oxidation occurs when the potato tissue is damaged upon invasion.

The phenol situation is interesting in fusarial wilt of tomato. One of the outstanding characteristics of this disease is the darkening of the vascular bundles. Davis *et al.* (1953) asked themselves, what is the color and how does it originate?

After studying the problem, they decided from their data that the pigment is melanoid, that it derives from a phenol which is oxidized to a quinone, and polymerized to a pigment. They decided that the phenol comes from a phenolic glycoside in the host, that the hydrolytic enzyme comes from the invading fungus, and that the phenoloxidase comes from the host. In brief, the course of events in blackening of the vascular bundle is that a phenol glucoside of the host is hydrolyzed by a fungus  $\beta$ -glucosidase, that the host enzymes oxidize the phenol to a quinone and convert the quinone to a pigment.

Presumably the same situation occurs in other wilt diseases, such as Dutch elm disease, where the vascular elements are discolored. Here, then, is a parallel situation to the potato scab. In potato scab the chlorogenic acid phenol that is liberated is somewhat fungicidal and its quinoidal analogue more so. Presumably, then, the phenols and the quinones that are precursors of the pigments in vascular diseases are fungitoxic also. The question is, do they discourage invasion?

Perhaps the Dutch elm disease might be considered first. We know that the so-called black strain of the Dutch elm disease fungus is a much more pathogenic strain than the light colored strain. Presumably, this can be explained by the results of Rich and Horsfall (1954). They found that *Monilinia fructicola*, a hyaline fungus, is poisoned by phenols and quinones while *Stemphylium sarcinaeforme*, a black fungus, generally is not. The black fungus is able to detoxicate phenols and quinones by converting them to bland black pigments, but the hyaline fungus cannot. Possibly, the hyaline strain of the Dutch elm disease fungus is unable to detoxicate the phenols or quinones formed upon invasion, and is thus poisoned. The black strain can detoxicate them, is not poisoned, and thus produces more damage than the hyaline strain. Similarly, Taylor and Decker (1947) report that the strains of the potato scab fungus that form black pigments are more pathogenic than hyaline strains.

One wonders how the blackening of the tomato xylem fits into the fusarial wilt picture. Apparently the fungus, being colored, can tolerate the phenols or the quinones that are precursors of the pigment. Perhaps the fungus can detoxicate the compounds as *Stemphylium sarcinaeforme* does. One observation is interesting. Gothoskar *et al.* (1955) have shown that respiratory inhibitors such as 2,4-dinitrophenol, sodium fluoride, thiourea, and sodium diethyldithiocarbamate break down the resistance of the Jefferson tomato to fusarial wilt. The last two named are active inhibitors of phenoloxidases. Perhaps these two, at least, prevent the formation of a toxic quinone and thus destroy natural resistance.

The case of verticillial wilt of tomato is interesting in this context. Here is a vascular disease in which the vascular system is only weakly discolored, if at all. The quinone or the melanin reaction must be blocked somewhere. This was mysterious until Caroselli (1955) discovered that *Verticillium* seems to excrete thiourea, at least in culture. If, indeed, it secretes thiourea in the tomato, it could inhibit the polyphenolase system and, thus, prevent the blackening. It probably also prevents the formation of the toxic quinone by the host and thus protects itself from damage. Stated in Gothoskar's terms, the thiourea secreted by the *Verticillium* fungus breaks down the host resistance.

The paper of Vörös *et al.* (1957) fits into an interesting niche here. Streptomycin is not fungitoxic but as a chemotherapeutant, it seems to increase the resistance of the potato to *Phytophthora infestans*. These authors give evidence that streptomycin activates the phenol oxidase system of the potato so that it produces more of the natural substances (possibly quinones) that are responsible for natural resistance. One wonders if it would not then increase the resistance of the potato to scab.

Certainly much more needs to be learned about the polyphenol



system. Apparently we can increase susceptibility by inhibiting it, decrease susceptibility by stimulating it.

This still leaves hanging the case of the tomato wilt disease. The quinone reaction does not seem to affect the situation in the normal infections. The Bonny Best variety is very susceptible, but the vascular bundles turn very dark. Here is an anomaly. The phenol  $\rightarrow$  quinone  $\rightarrow$  pigment reaction exists, but the fungus is not poisoned. This cannot be a thiourea case, either, because this would prevent coloration.

We can marshal what evidence we have. Davis *et al.* (1953) showed that *Fusarium oxysporum* f. *lycopersici* can hydrolyze phenol glycosides and use them as sources of carbon. Presumably, this suggests that the phenol formed in the tomato stem is not toxic to the fungus. The phenol apparently is oxidized by the host to a quinone. We do not know whether it is toxic to *Fusarium* or not. From their colors, we know that *Fusaria* can produce many pigments. Probably, *F. lycopersici* can detoxicate a quinone by forming a pigment, as *Stemphylium sarcinaeforme* does.

The results of Dimond and his colleagues (Davis and Dimond, 1953) on phenoxy and naphthoxy acetic acid analogues seem to find a place somewhere in this area of the reasoning. Essentially, all of these compounds inhibit the blackening reaction in tomato stems that have been inoculated with *Fusarium*. On this basis, the compounds have been rated as chemotherapeutic. They do indeed knock out the commonest symptom of disease.

We are unable to discover any other work on the effect of these compounds to inhibit the polyphenolase blackening reaction of higher plants. Several authors, however, report that 2,4-D inhibits pigmentation in dark-colored fungi, as for example in *Helminthosporium victoriae* (Bever and Slife, 1948).

It seems reasonably safe to assume then that these compounds do inhibit pigment formation. Do they inhibit quinone formation as well? We have no evidence here. The quinone may or may not be important, because the fungus does seem to be able to cope with it in nature.

One wonders what effect the phenoxy compounds would have on the resistant Jefferson variety of tomato. One wonders if the effect would be similar to that of the inhibitors of polyphenolase as reported by Gothoskar *et al.* (1955). Whether they act on the quinone or not, the fungus is not killed by them because when medication is withdrawn for a few days, the tomato dies from its infection.

These substances are phenol analogues. Possibly they inhibit blackening by acting as phenolic antimetabolites. This brings to mind another interesting result that has occurred in Dimond's tests. 2,4-Dichlorophenoxypropionic acid is not as effective in reducing the blackening reaction

as 2,4-dichlorophenoxyacetic acid. A similar result has been shown on chocolate spot of bean by Crowdy and Wain (1950). This disease also is read in terms of the blackening reaction. The propionic acid derivative does not reduce the blackening. The acetic acid derivative does.

According to Synerholm and Zimmerman (1947), the propionic acid analogue is degraded to a phenol by the host, the acetic acid analogue is not. Thus, the former would add to the blackening, if anything, the latter would not.

What, then, about other symptoms of disease? What about wilting? Generally, the growth substances reduce wilting too. However, Dimond has told us verbally of a compound which prevents the blackening of the vascular bundles but which permits 73% as much wilting as the check. This compound is  $2,4\text{-Cl}_2\text{C}_6\text{H}_5\text{—O—(CH}_2\text{—CH}_2\text{—O)}_2\text{—CH}_2\text{—CH}_2\text{OH}$ . A homologue with four ethoxy groups in the side chain gave similar results. It is obvious that these two compounds can eliminate one symptom of tomato wilt, but not others.

## 2. Synthetic Therapeutants

Until the modern revival of interest in synthetic therapeutants, most of the cases of successful cure of plant disease were limited to local or topical chemotherapy. Apple scab lesions could be cured with lime sulfur and organic mercurials. Certain cereal smuts could be cured by treating the infected seeds with organic mercurials, but we probably had no translocatable "systemic fungicide."

By our definition, a systemic fungicide is one that distributes itself systemically through the plant and kills the pathogen by direct toxic action. Crowdy and Wain (1950) spoke of the systemic fungicidal activity of analogues of phenoxyacetic acid. These compounds are only weakly fungitoxic, however. This led Horsfall and Dimond (1951) to suggest that they probably act to make the host resistant rather than to kill out the pathogen. Later researches seem to bear this out (Davis and Dimond, 1952).

Crowdy and Davies (1952) showed that phenyl mercury acetate is translocated from the roots to leaves of bean plants and inhibits the chocolate spot disease.

Antibiotics probably showed the first really good case of a systemic therapeutant that could indeed kill out the invader. Now we know that cycloheximide possesses such properties for wheat rust (Wallen and Millar, 1957) and cherry leaf spot, cycloheximide semicarbazone for *Coccomyces hiemalis* in sour cherry (Hamilton and Szkolnik, 1958), pyridine-2-thiol-*N*-oxide for cucumber scab (Sander and Allison, 1956, and Rombouts and Sijpesteijn, 1958), 4-nitrosopyrazole for *Alternaria* on

tomato (McNew and Sundholm, 1949), and griseofulvin for several diseases.

The antifungal polypeptide, "Phytoactin," has markedly reduced infection with oak wilt when applied in solution through trunk cuts to northern pin oaks (Phelps *et al.*, 1957). In University of Wisconsin experiments on oaks, 3 to 10 inches in diameter and 20 to 40 feet in height, oligomycin yielded an average of 13% healthy trees, Actidione 20%, its acetate derivative 30% for three seasons, and Phytoactin 40% after two seasons. By comparison, 95% of the untreated, inoculated check trees had died. A group of inoculated greenhouse and nursery oaks in Ohio tests were not benefited.

A few reports suggest that viruses can be inactivated in plants. Stoddard (1947) reported curing peach seedlings of X-disease by injecting the outer ends of the stems with calcium chloride and zinc sulfate. The zinc effect has by now been investigated for numerous virus diseases. It seems to be effective for some such as carnation mosaic (Thomas and Baker, 1949) but not for others such as tobacco mosaic virus on *Nicotiana glutinosa* (Yarwood, 1954).

Respiration inhibitors seem to inhibit virus multiplication (Leben and Fulton, 1951). Similarly, some antimetabolites seem to be effective for viruses, as for instance sulfanilamide (Stoddard, 1947), thiouracil (Commoner and Mercer, 1951), and guanazolo (Matthews, 1951). The inhibition of virus multiplication is treated extensively in this treatise by Matthews in Chapter 12 of Volume II.

Therapeutic action of sulfonamide and related sulfa compounds is currently believed to be by interference with enzymes necessary for growth. They disturb the utilization of para-aminobenzoic acid and, hence, inhibit the action of certain coenzymes essential for bacterial and actinomycete growth. A possibility for curing systemic bacterial diseases is the internal application of translocatable sulfones capable of breaking down *in vivo* into diamino-diphenyl sulfone which exercises antibiotic action similar to the sulfonamides.

### 3. Possibilities for a Tailor-Made Therapeutant

Perhaps something can be derived from our experience to date to suggest possibilities to make better therapeutants. As we have seen, phenols and quinones seem to be involved in natural host resistance. It seems probable that our knowledge of these compounds will increase.

The evidence is fairly clear from Allen's review (Chapter 12 of this volume) and from the work of Davis *et al.* (1953) that in the very act of invasion phenols may be freed from natural nontoxic compounds such as phenolic glycosides.

This suggests that we feed such detoxicated phenols to the host. They should not be phytotoxic. They would lie in wait for an invading microbe which would free them from their bound form. Thus, the microbe would poison itself at the point of invasion. In effect, this would be a case of synthetic hypersensitivity which is discussed by Müller in Chapter 13 of this volume.

The work of Byrde and Woodcock (1952) suggests a device that would work on this principle. 2,4-Dichloronaphthoquinone is a powerful fungicide used commercially as a protectant. Byrde and Woodcock reduce it to the dihydroxynaphthalene form and then acetylate the two phenolic groups. This procedure renders the compound nontoxic to apple foliage when applied to the surface.

The apple scab fungus, *Venturia inaequalis*, can deacetylate the compound by means of a fungus esterase. According to Byrde and Woodcock, the dihydroxynaphthalene thus liberated is toxic. Horsfall (1956) suggested that the compound is probably oxidized by the fungus back to the still more toxic naphthoquinone.

If, now, the diacetoxyl derivative were used as a chemotherapeutant, it should be the answer to our prayers. The invading fungus should free the phenolic form, the host should convert it to a quinone. It cannot be converted to a pigment because the chlorine blocks the important position. Thus, it should be an ideal chemotherapeutant.

The concept is too simple. Upon trial we find that the host esterases can liberate the phenol and oxidize it as readily as those of the fungus. The host dies by its own hand even before the pathogen arrives. It works on the surface of an apple leaf, but not inside the tissue.

Other acetylated phenols have followed this one down the drain. Esterases for acetic acid derivatives seem to be too common in nature. Perhaps, we need to investigate other acids, such as benzoic. The linkage here is harder to break according to Byrde and Woodcock (1956), but on the other hand the fungus esterases may not break it either.

An interesting ester is the thioglycolic ester of ethylenedisithiocarbamic acid as proposed by van der Kerk (1956) as a systemic chemotherapeutant. According to van der Kerk the host deesterifies this linkage and recovers the nabam itself.

Perhaps phosphate or borate esters would work better. The phosphates have solved the problem for systemic insecticides.

Perhaps amide linkages are better sources of detoxication. Whether fungus amidases are more common or more specific than host amidases, can only be settled by empirical attack.

In any case, this appears to be a lucrative source of possible chemotherapeutants that might act differentially on host and pathogen.



### C. Development of Resistance to Chemotherapeutants

As soon as one speaks of chemically killing a pathogen, one cannot avoid the possible development of resistance by the pathogen. This problem has plagued our medical colleagues almost from the day of the development of the first successful chemotherapeutant.

Fortunately, it has not been a serious problem for the plant pathologist, perhaps because we do not yet have a chemotherapeutant in wide-scale commercial use. The problems of acquired resistance are treated by Buxton in Chapter 10 of Volume II.

Acquired resistance to chemotherapeutants has been reported for bacteria. Mitchell *et al.* (1952) report that *Xanthomonas phaseoli* causing bean blight develops resistance to streptomycin. Strangely enough the halo blight bacterium on beans, *Pseudomonas phaseolicola*, does not develop resistance so readily. *Erwinia amylovora* on apple and *Xanthomonas vesicatoria* on pepper also rapidly develop resistance to streptomycin.

Our colleagues in medicine solve the resistance problem in part, at least, by using mixtures of antibiotics. English and van Halsema (1954) have shown that plant bacteria also develop resistance less rapidly if more than one antibiotic is used.

### D. Increasing Host Resistance

Increasing natural resistance to development of the pathogen by chemical modification of the host cell is possible. Weintraub *et al.* (1952) suggest that the antiviral effect of Stoddard's (1947) zinc sulfate is probably a case of increasing host resistance. Chemotherapy may also be used to induce extreme susceptibility or hypersensitivity. This phenomenon of hypersensitivity occurs naturally between wheats and the rust fungus, potatoes and the late blight fungus, etc.

#### 1. Effect of Auxins

Plant growth regulators give striking effects in the amelioration of some diseases. These effects were discovered independently and reported first by Crowdy and Wain (1950) for the chocolate spot of beans and then by Dimond and Chapman (1951) for fusarial wilt of tomatoes. More recently they have been tested by Beckman (1958) for Dutch elm disease.

These compounds were selected originally for testing because they were known to be translocated.

Dimond and his group continued their researches on the auxins and

auxin-like substances and found that almost any auxin that acts on the tomato reduces the fusarial wilt syndrome (Davis and Dimond, 1953).

The striking feature, however, is that the fungitoxicity of auxins is extremely low, so low in fact that it could not possibly account for the reduction in disease (Davis, 1952).

This discovery led to the conclusion that the auxins somehow make the plant resistant to disease. The nature of this mechanism is still vague, but some progress is being made. The final solution must await information on just what the auxins do and can do to the plant.

## *2. Relation to Sugars*

In the meantime, a few statements can be made. For one thing, auxins exert a strong influence on the sugar levels in plants and Horsfall (1956) hazarded a conjecture that the sugar levels are correlated with disease.

This phase of the matter has been pursued in considerably more detail by Horsfall and Dimond (1957).

The effect of auxins on sugar levels varies with the time that elapses after treatment. Usually the sugar rises at first and then falls. Maleic hydrazide affects the phloem and reduces translocation of sugars from the leaves. Hence, it usually raises the sugar in the leaves, reduces it in stems and roots.

Diseases may be high sugar diseases or low sugar diseases, that is, encouraged by high sugar or discouraged by high sugar. Rusts, powdery mildews, and chocolate spot of broad bean are high sugar diseases. They should be reduced by 2,4-D and increased by maleic hydrazide, and they are.

On the other hand, helminthosporial and alternarial leaf spots and anthracnoses seem to be low sugar diseases. 2,4-D increases them.

Fusarial wilt seems also to be a low sugar disease; 2,4-D decreases it at first when stem sugars are high, but the effect wears off as sugar levels fall. Maleic hydrazide, on the other hand, increases fusarial wilt (Waggoner and Dimond, 1957), presumably because maleic hydrazide reduces stem sugars.

We have difficulty in believing that the sugar effects can stand alone. Possibly the sugar effect is direct on the high sugar diseases. In this case sugar reduction could be directly related to disease reduction. The effect on low sugar diseases is less easy to follow. It seems probable that the sugar is a marker for something else. The pathogens that induce low sugar disease are sugar feeders just like the rusts and powdery mildews. It seems likely that low sugar must be correlated with low something

else, and that the low something else is related to low resistance. We must search for the "something else" that might account for resistance to *Fusarium* and to the leaf spots mentioned.

Growth hormones generally increase the resistance of tomato plants to fusarial wilt. For a series of related growth regulants, Corden and Dimond (1959) studied the types of growth hormone activity with which chemotherapeutic activity is best correlated. They showed that the ability of compounds to inhibit root elongation is well correlated. The relation of this hormone function to the nature of pectin composition of the host plant is suggested by this study.

Various chemicals of therapeutic promise against the Dutch elm disease are antagonistic to indoleacetic acid as judged by the pea epicotyl elongation test (Beckman, 1958). A good correlation was found between the inhibition of springwood development and suppression of symptoms.

And thus we end our story. This is where our ideas of "Plant Disease Therapy" stand today. But, tomorrow—who knows?

#### REFERENCES

- Anderson, H. W., and I. Nienow. 1947. Effect of streptomycin on higher plants. *Phytopathology* **37**: 1.
- Anonymous. 1955. Progress in the fight against Dutch elm disease. *Rhode Island Agr.* **2**: 1-4.
- Ark, P. A. 1941. Chemical eradication of crown gall on almond trees. *Phytopathology* **31**: 956-957.
- Ark, P. A., and S. M. Alcorn. 1956. Antibiotics as bactericides and fungicides against diseases of plants. *Plant Disease Reprtr.* **40**: 85-92.
- Bartholomew, E. T. 1928. Internal decline (endoxerosis) of lemons. VI. Gum formation in the lemon fruit and its twig. *Am. J. Botany* **15**: 548-563.
- Beckman, C. H. 1958. Growth inhibition as a mechanism in Dutch elm disease therapy. *Phytopathology* **48**: 172-176.
- Beckman, C. H. 1959. Dutch elm disease control with polychlorobenzoic acid. *Phytopathology* **49**: (In press).
- Bever, W. M., and F. W. Slife. 1948. Effect of 2,4-D in culture medium on the growth of three pathogenic fungi. *Phytopathology* **38**: 1038.
- Braun, A. C. 1950. The mechanism of action of a bacterial toxin on plant cells. *Proc. Natl. Acad. Sci. U. S.* **36**: 423-427.
- Brian, P. W. 1952a. Antibiotics as systemic fungicides and bactericides. *Ann. Appl. Biol.* **39**: 434-438.
- Brian, P. W. 1952b. Systemic fungicides. *Plant Protect. Overseas Rev.* **3**(3): 5-10.
- Brian, P. W., J. M. Wright, J. Stubb, and A. M. Way. 1951. Uptake of antibiotic metabolites of soil micro-organisms by plants. *Nature* **167**: 347-349.
- Brierley, P., and F. F. Smith. 1957. Symptoms of chrysanthemum flower distortion, dodder transmission of the virus, and heat cure of infected plants. *Phytopathology* **47**: 448-450.
- Byrde, R. J. W., and D. Woodcock. 1952. Fungicides and phytotoxicity. *Nature* **169**: 503-504.

- Byrde, R. J. W., and D. Woodcock. 1956. Fungicidal activity and chemical constitution. III. Pentachlorophenol derivatives. *Ann. Appl. Biol.* **44**: 138-144.
- Caroselli, N. E. 1955. The relation of soil-water content and that of sapwood-water to the incidence of maple wilt caused by *Verticillium* sp. *Phytopathology* **45**: 184.
- Caroselli, N. E. 1957. *Verticillium* wilt of maples. *Rhode Island Univ. Agr. Expt. Sta. Bull.* **335**: 1-84.
- Chapman, R. A. 1951. Relation of specific chemotherapeutants to the infection court. *Phytopathology* **41**: 6-7.
- Chen, C. C. 1920. Internal fungous parasites of agricultural seeds. *Maryland Univ. Agr. Expt. Sta. Bull.* **240**: 81-110.
- Clayton, E. E. 1937. Water soaking of leaves in relation to development of the blackfire disease of tobacco. *J. Agr. Research* **55**: 883-889.
- Commoner, B., and F. Mercer. 1951. Inhibition of the biosynthesis of tobacco mosaic virus by thiouracil. *Nature* **168**: 113-114.
- Corden, M. E., and A. E. Dimond. 1959. The effect of growth substances on disease resistance and plant growth. *Phytopathology* **49**: (In press).
- Crafts, A. S. 1948. A theory of herbicidal action. *Science* **108**: 85-86.
- Crowdy, S. H. 1952. The chemotherapy of plant disease. *Empire J. Exptl. Agr.* **20**: 187-194.
- Crowdy, S. H. 1953. Observations on the effect of growth-stimulating compounds on the healing of wounds on apple trees. *Ann. Appl. Biol.* **40**: 197-207.
- Crowdy, S. H. 1957. The uptake and translocation of griseofulvin, streptomycin, and chloramphenicol in plants. *Ann. Appl. Biol.* **45**: 208-215.
- Crowdy, S. H., and M. E. Davies. 1952. Studies on systemic fungicides. II. Behavior of groups of reported chemotherapeutants. *Phytopathology* **42**: 127-131.
- Crowdy, S. H., and D. Pramer. 1955a. Movement of antibiotics in higher plants. *Chem. & Ind. (London)* **1955**: 160-162.
- Crowdy, S. H., and D. Pramer. 1955b. The occurrence of translocated antibiotics in expressed plant sap. *Ann. Botany (London)* [N.S.] **19**: 79-86.
- Crowdy, S. H., and D. Rudd Jones. 1956. Partition of sulphonamides in plant roots: A factor in their translocation. *Nature* **178**: 1165-1167.
- Crowdy, S. H., and R. L. Wain. 1950. Aryloxyaliphatic acids as systemic fungicides. *Nature* **165**: 937-938.
- Crowdy, S. H., J. F. Grove, H. G. Hemming, and K. C. Robinson. 1956. The translocation of antibiotics in higher plants. II. The movement of griseofulvin in broad bean and tomato. *J. Exptl. Botany* **7**: 42-64.
- Curtis, L. C. 1943. Deleterious effects of guttated fluids on foliage. *Am. J. Botany* **30**: 778-781.
- Davis, D. 1952. Chemotherapeutic activity may be independent of fungitoxicity. *Phytopathology* **42**: 6.
- Davis, D., and A. E. Dimond. 1952. Altering resistance to disease with synthetic organic chemicals. *Phytopathology* **42**: 563-567.
- Davis, D., and A. E. Dimond. 1953. Inducing disease resistance with plant growth-regulators. *Phytopathology* **43**: 137-140.
- Davis, D., and J. W. Rothrock. 1956. Localized systemic activity of griseofulvin in the control of Alternaria blight to tomato. *Plant Disease Repr.* **40**: 328-331.
- Davis, D., P. E. Waggoner, and A. E. Dimond. 1953. Conjugated phenols in the *Fusarium* wilt syndrome. *Nature* **172**: 959-961.
- Dimond, A. E. 1959. Plant chemotherapy. In "Plant Pathology-Problems and Progress, 1908-1958." Univ. Wisconsin Press, Wisconsin. (In press).



- Dimond, A. E., and R. A. Chapman. 1951. The chemotherapeutic properties of two compounds against *Fusarium* wilts. *Phytopathology* **41**: 11.
- Dimond, A. E., and J. G. Horsfall. 1959. Plant Chemotherapy. *Ann. Rev. Plant Physiol.* **10**: (In press).
- Dimond, A. E., and P. E. Waggoner. 1953. On the nature and role of vivotoxins in plant disease. *Phytopathology* **43**: 229-235.
- Dimond, A. E., D. Davis, R. A. Chapman, and E. M. Stoddard. 1952. Plant chemotherapy as evaluated by the *Fusarium* wilt assay on tomatoes. *Conn. Agr. Expt. Sta. (New Haven) Bull.* **557**: 1-82.
- Dubos, R. J. 1945. "The Bacterial Cell." Harvard Univ. Press, Cambridge, Massachusetts. 460 pp.
- Dubos, R. J. 1958. Pasteur and Modern Science. In "The Pasteur Fermentation Centennial 1857-1957." Chas. Pfizer and Co., Inc., New York. 207 pp.
- Dye, M. H. 1956. Studies on the uptake and translocation of streptomycin by peach seedlings. *Ann. Appl. Biol.* **44**: 567-575.
- Ehrlich, P. 1913. Chemotherapeutics: scientific principles, methods, and results. *Lancet* **2**: 445-451.
- English, A. R., and G. van Halsema. 1954. A note on the delay in the emergence of resistant *Xanthomonas* and *Erwinia* strains by the use of streptomycin plus terramycin combinations. *Plant Disease Reprtr.* **38**: 429-431.
- Erdtman, H. 1949. Heartwood extractives of conifers. Their fungicidal and insect-repellent properties and taxonomic interest. *Tappi* **32**: 305-310.
- Feldman, A. W., N. E. Caroselli, and F. L. Howard. 1950. Physiology of toxin production by *Ceratostomella ulmi*. *Phytopathology* **40**: 341-354.
- Fulton, H. R., and W. W. Coblentz. 1929. The fungicidal action of ultra-violet radiation. *J. Agr. Research* **38**: 159-168.
- Goodman, R. N. 1954. Development of methods for use of antibiotics to control fireblight (*Erwinia amylovora*). *Missouri Univ. Agr. Expt. Sta. Research Bull.* **540**: 1-16.
- Gopalkrishnan, K. S., and J. A. Jump. 1952. The antibiotic activity of thiolutin in the chemotherapy of the *Fusarium* wilt of tomato. *Phytopathology* **42**: 338-339.
- Gothoskar, S. S., R. P. Scheffer, M. A. Stahmann, and J. C. Walker. 1955. Further studies on the nature of *Fusarium* resistance in tomato. *Phytopathology* **45**: 303-307.
- Gottlieb, D. 1957. The effect of metabolites on antimicrobial agents. *Phytopathology* **47**: 59-67.
- Gray, R. A. 1955. Increasing the effectiveness of streptomycin against the common blight of beans with glycerin. *Plant Disease Reprtr.* **39**: 567-568.
- Gray, R. A. 1958. The downward translocation of antibiotics in plants. *Phytopathology* **48**: 71-78.
- Halleck, F. E., and V. W. Cochrane. 1950. The effect of fungistatic agents on the bacterial flora of the rhizosphere. *Phytopathology* **40**: 715-718.
- Hamilton, J. M., and M. Szkolnik. 1958. Control of *Coccomyces hiemalis* by systemic movement of cycloheximide semi-carbazone in sour cherry following root or leaf absorption. *Phytopathology* **48**: 262.
- Hidaka, Z., and H. Murano. 1956. Studies on the streptomycin for plants. I. Behavior of *Pseudomonas solanacearum* and *Ps. tabaci* treated with streptomycin *in vitro* and surface absorption of streptomycin in the plant. *Ann. Phytopathol. Soc. Japan* **20**: 143-147.
- Hilborn, M. T. 1953. Effect of various chemicals on infection by *Rhizoctonia solani* and *Verticillium albo-atrum*. *Phytopathology* **43**: 475.
- Hopper, B. E., and A. C. Tarjan. 1954. Chlorophenyl methyl rhodanine: Its mode of action against root nematode infection. *Plant Disease Reprtr.* **38**: 542-544.

- Horsfall, J. C. 1956. "Principles of Fungicidal Action." *Chronica Botanica*, Waltham, Massachusetts. 280 pp.
- Horsfall, J. G., and A. E. Dimond. 1951. Plant chemotherapy. *Trans. N. Y. Acad. Sci.* **13**: 338-341.
- Horsfall, J. G., and A. E. Dimond. 1957. Interactions of tissue sugar, growth substances, and disease susceptibility. *Z. Pflanzenkrankh. u. Pflanzenschutz* **64**: 415-421.
- Horsfall, J. G., and G. A. Zentmyer. 1942. Antidoting the toxins of plant diseases. *Phytopathology* **32**: 22-23.
- Howard, F. L. 1941. Antidoting toxin of *Phytophthora cactorum* as a means of plant disease control. *Science* **94**: 345.
- Howard, F. L., and M. B. Sorrell. 1943. Cationic phenyl mercury compounds as specific apple-scab eradicates on foliage. *Phytopathology* **33**: 1114.
- Jeffreys, E. G. 1952. The stability of antibiotics in soils. *J. Gen. Microbiol.* **7**: 295-312.
- Lakshminarayanan, K., and D. Subramanian. 1955. Is fusaric acid a vivotoxin? *Nature* **176**: 697-98.
- Leben, C., and R. W. Fulton. 1951. The inhibition of virus symptom expression by sodium azide, potassium cyanide, and two antibiotics. *Phytopathology* **41**: 23.
- Lemin, A. J., and W. E. Magee. 1957. Degradation of cycloheximide derivatives in plants. *Plant Disease Reptr.* **41**: 447-448.
- McMurtrey, J. E., Jr. 1948. Visual symptoms of malnutrition in plants. In "Diagnostic Techniques for Soils and Crops." Am. Potash Institute, Washington, D. C. 308 pp.
- McNew, G. L., and N. K. Sundholm. 1949. The fungicidal activity of substituted pyrazoles and related compounds. *Phytopathology* **39**: 721-751.
- Martin, H. 1950. Advances in chemical methods of crop protection. *J. Sci. Food Agr.* **1**: 163-167.
- Martin, M., and D. Gottlieb. 1955. The production and role of antibiotics in soil. V. Antibacterial activity of five antibiotics in the presence of soil. *Phytopathology* **45**: 407-408.
- Masé, P. 1914. Recherches de physiologie végétale. IV. Influences respectives des éléments de la solution minérale sur le développement du maïs. *Ann. inst. Pasteur* **28**: 21-68.
- Matthews, R. E. F. 1951. Effect of some substituted purines on the development of plant virus infections. *Nature* **167**: 892-893.
- Mitchell, J. W., W. J. Zaunmeyer, and W. P. Anderson. 1952. Translocation of streptomycin in bean plants and its effect on bacterial blights. *Science* **115**: 114-116.
- Mitchell, J. W., W. J. Zaunmeyer, and W. H. Preston, Jr. 1953. Movement of streptomycin in bean plants. *Phytopathology* **43**: 480.
- Mosca, A. 1958. "L'Atomo in Agricoltura." Cuneo, Italy. 48 pp.
- Müller, A. 1926. Die innere Therapie der Pflanzen. *Monograph angew. Entomol.* No. 8. Paul Parey, Berlin.
- Napier, E. J., D. I. Turner, A. Rhodes, and J. P. R. Toothill. 1956. The systemic action against *Pseudomonas medicaginis* var. *phaseolicola* of a streptomycin spray applied to dwarf beans. *Ann. Appl. Biol.* **44**: 145-151.
- Phelps, W. R., J. E. Kuntz, and A. J. Riker. 1957. Antibiotics delay oak wilt symptoms on inoculated northern pin oaks in central Wisconsin. *Phytopathology* **47**: 27.
- Pramer, D. 1953. Observations on the uptake and translocation of five actinomycete antibiotics by cucumber seedlings. *Ann. Appl. Biol.* **40**: 617-622.

- Pramer, D. 1954. The movement of chloramphenicol and streptomycin in broad bean and tomato plants. *Ann. Botany (London)* **18**: 463-470.
- Prescott, G. C., H. Emerson, and J. H. Ford. 1956. Determination of cycloheximide residues in cherries. *J. Agr. Food Chem.* **4**: 343-345.
- Prévost, B. 1807. Mémoire sur la cause immédiate de la carie ou charbon des blés, et de plusieurs autres maladies des plantes, et sur les préservatifs de la carie. *Phytopathol. Classics* **6**: 94 pp. (Transl. by G. W. Keitt in 1939.)
- Reed, H. S., and J. Dufrenoy. 1935. The effects of zinc and iron salts on the cell structure of mottled orange leaves. *Hilgardia* **9**: 113-135.
- Rennerfelt, E. 1945. The influence of the phenolic compounds in the heartwood of Scots pine (*Pinus silvestris* L.) on the growth of some decay fungi in nutrient solution. *Svensk Botan. Tidskr.* **39**: 311-318.
- Rich, S. 1956. Foliage fungicides plus glycerin for the chemotherapy of cucumber scab. *Plant Diseases Reprtr.* **40**: 620-621.
- Rich, S., and J. G. Horsfall. 1954. Relation of polyphenol oxidases to fungitoxicity. *Proc. Natl. Acad. Sci. U. S.* **40**: 139-145.
- Riere, J. 1940. Molds: development, identification, prevention. *Am. Dyestuff. Reprtr.* **29**: 211-212.
- Roach, W. A. 1939. Plant injection as a physiological method. *Ann. Botany (London) [N.S.]* **3**: 155-226.
- Robison, R. S., R. L. Starkey, and O. W. Davidson. 1954. Control of bacterial wilt of chrysanthemums with streptomycin. *Phytopathology* **44**: 646-650.
- Rombouts, J. E., and A. K. Sijpesteijn. 1958. The chemotherapeutic effect of pyridine-2-thiol-N-oxide and some of its derivatives on plant diseases. *Ann. Appl. Biol.* **46**: 30-36.
- Rudd Jones, D., and J. Wignall. 1955. Acetylation of sulphanilamide in plants. *Nature* **175**: 207-208.
- Sander, E., and P. Allison. 1956. Bioassay of the translocated fungicide, 2-pyridinethiol-1-oxide, in cucumber seedlings. *Phytopathology* **46**: 25.
- Sanwal, B. D. 1956. Investigations on the metabolism of *Fusarium lycopersici* Sacc. with the aid of radioactive carbon. *Phytopathol. Z.* **25**: 333-384.
- Schaal, L. A., and G. Johnson. 1955. The inhibitory effect of phenolic compounds on the growth of *Streptomyces scabies* as related to the mechanism of scab resistance. *Phytopathology* **45**: 626-628.
- Shaw, L. 1935. Intercellular humidity in relation to fire blight susceptibility in apple and pear. New York *Cornell Univ. Agr. Exptl. Sta. Mem.* **181**: 1-40.
- Shaw, L., G. B. Lucas, and G. F. Thorne, Jr. 1957. Further studies with streptomycin alone and in combination with other chemicals for wildfire control in burley tobacco plant beds, North Carolina, 1956. *Plant Disease Reprtr.* **41**: 99-102.
- Shear, G. M. 1936. Lanolin as a wound dressing for trees. *Proc. Am. Soc. Hort. Sci.* **34**: 286.
- Siminoff, P., and D. Gottlieb. 1951. The production and role of antibiotics in the soil: I. The fate of streptomycin. *Phytopathology* **41**: 420-430.
- Stoddard, E. M. 1946. Soil applications of oxyquinolin benzoate for the control of foliage wilting in elms caused by *Graphium ulmi*. *Phytopathology* **36**: 682.
- Stoddard, E. M. 1947. X-disease of peach and its chemotherapy. *Conn. Agr. Expt. Sta. (New Haven) Bull.* **506**: 1-19.
- Stoddard, E. M. 1954. Chemotherapeutic control of cucumber scab. *Phytopathology* **44**: 507.

- Stoddard, E. M., and A. E. Dimond. 1949. The chemotherapy of plant diseases. *Botan. Rev.* **15**: 345-376.
- Stokes, A. 1954. Uptake and translocation of griseofulvin by wheat seedlings. *Plant and Soil* **5**: 132-142.
- Strong, F. C., and D. Cation. 1940. Control of cedar rust with sodium dinitro-cresylate. *Phytopathology* **30**: 983.
- Subramanian, D. 1956. Role of trace element chelation in the *Fusarium* wilt of cotton. *Proc. Indian Acad. Sci. Sect. B*, **43**: 302-307.
- Synerholm, M. E., and P. W. Zimmerman. 1947. Preparation of a series of *w*-(2,4-dichlorophenoxy)-aliphatic acids and some related compounds with a consideration of their biochemical role as plant-growth regulators. *Contribs. Boyce Thompson Inst.* **14**: 369-382.
- Taylor, C. F., and P. Decker. 1947. A correlation between pathogenicity and cultural characteristics in the genus *Actinomyces*. *Phytopathology* **37**: 49-58.
- Taylor, G. A., and C. B. Smith. 1957. The use of plant analysis in the study of blossom-end rot of tomato. *Proc. Am. Hort. Sci.* **70**: 341-349.
- Thomas, W. D., and R. R. Baker. 1949. Chemical inactivation of the carnation mosaic virus *in vivo*. *J. Colo.-Wyo. Acad. Sci.* **4**: 51.
- Tisdale, W. H., and W. N. Cannon. 1929. Ethyl mercury chloride as a seed grain disinfectant. *Phytopathology* **19**: 80.
- Turner, R., and W. W. McCall. 1957. Studies on crop responses to molybdenum and lime in Michigan. *Mich. State Univ. Agr. Expt. Sta. Quart. Bull.* **40**: 268-281.
- Tyner, L. E. 1957. Factors influencing the elimination of loose smut from barley by water-soak treatment. *Phytopathology* **47**: 420-422.
- van der Kerk, G. J. M. 1956. The present state of fungicide research. *Mededel. Landbouwhogescholen Opzoekingsstat. Staat Gent.* **21**: 305-339.
- Van Raalte, M. H. 1952. A test for the translocation of fungicides through plant tissues. *Proc. 3rd Intern. Congr. Crop Protect., Paris, 1952* pp. 76-78.
- Vörös, J., Z. Király, and G. L. Farkas. 1957. Role of polyphenolase in streptomycin-induced resistance to *Phytophthora* in potato. *Science* **126**: 1178.
- Waggoner, P. E. 1955. Radiation and resistance of tubers to rot. *Am. Potato J.* **32**: 448-450.
- Waggoner, P. E., and A. E. Dimond. 1952. Crown gall suppression by ionizing radiation. *Am. J. Botany* **39**: 679-684.
- Waggoner, P. E., and A. E. Dimond. 1955. Assaying effect of growth regulators upon plant tumors. *Conn. Agr. Expt. Sta. (New Haven) Bull.* **587**.
- Waggoner, P. E., and A. E. Dimond. 1956. Altering disease resistance with ionizing radiation. *Phytopathology* **46**: 125-127.
- Waggoner, P. E., and A. E. Dimond. 1957. Altering disease resistance with ionizing radiation and growth substances. *Phytopathology* **47**: 125-130.
- Walker, A. T., and F. G. Smith. 1952. Effect of actidione on growth and respiration of *Myrothecium verrucaria*. *Proc. Soc. Exptl. Biol. Med.* **81**: 556-559.
- Walker, J. C., K. P. Link, and H. R. Angell. 1929. Chemical aspects of disease resistance in the onion. *Proc. Natl. Acad. Sci. U. S.* **15**: 845-850.
- Wallen, V. R., and R. L. Millar. 1957. The systemic activity of cycloheximide in wheat seedlings. *Phytopathology* **47**: 291-294.
- Weintraub, M., J. D. Gilpatrick, and R. S. Willison. 1952. The effect of certain water-soluble compounds on virus infection. *Phytopathology* **42**: 417-419.



- Wellman, F. L. 1950. Dissemination of *Omphalia* leaf spot of coffee. *Turrialba* **1**: 12-27.
- Woolley, D. W., R. B. Pringle, and A. C. Braun. 1952. Isolation of the phytopathogenic toxin of *Pseudomonas tabaci*, an antagonist of methionine. *J. Biol. Chem.* **197**: 409-417.
- Wright, J. M., and J. F. Grove. 1957. The production of antibiotics in soil. V. Break-down of griseofulvin in soil. *Ann. Appl. Biol.* **45**: 36-43.
- Yarwood, C. E. 1954. Zinc increases susceptibility of bean leaves to tobacco mosaic virus. *Phytopathology* **44**: 230-233.
- Yarwood, C. E. 1955. Therapeutic action of sulfur for powdery mildews and rusts. In "Therapy of Fungus Diseases" (T. H. Sternberg and V. D. Newcomber, eds.), Little, Brown, Boston, Massachusetts. 337 pp.
- Zentmyer, G. A. 1942. Toxin formation and chemotherapy in relation to Dutch elm disease. *Phytopathology* **32**: 20.
- Zentmyer, G. A. 1954. Chemotherapy for control of *Phytophthora* root rot of avocado. *Phytopathology* **44**: 511.
- Zentmyer, G. A., J. G. Horsfall, and P. P. Wallace. 1946. Dutch elm disease and its chemotherapy. *Conn. Agr. Expt. Sta. (New Haven) Bull.* **498**: 1-70.

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